THE AMERICAN JOURNAL *of* HUMAN GENETICS

THE AMERICAN JOURNAL *of* HUMAN GENETICS

Edited by

ARTHUR G. STEINBERG

in collaboration with

HORACE W. NORTON
NEWTON E. MORTON
HERMAN M. SLATIS
JOHN B. GRAHAM
F. CLARKE FRASER
VICTOR A. MCKUSICK

Volume 12
1960

Published Quarterly by
AMERICAN SOCIETY OF HUMAN GENETICS

Printed at the WAVERLY PRESS INC.
Mt. Royal & Guilford Aves.
Baltimore 2, Maryland

CONTENTS

VOL. 12, NO. 4, DECEMBER, 1960

PART 1 (OF 2 PARTS)

VOL. 12, NO. 4, DECEMBER, 1960

PART 2 (OF 2 PARTS)

The American Journal of Human Genetics

Vol. 12 No. 1 *March 1960*

A Study of Retinoblastoma in Ohio

MADGE T. MACKLIN

Department of Medicine, Ohio State Health Center, Columbus, Ohio

INTRODUCTION

In October 1955, an investigation into the frequency of retinoblastoma in Ohio was started. The purpose was fourfold: to find the frequency of the disease; to determine, as closely as possible, the mutation rate; to test the existing estimates of the degree of penetrance; and to express some sort of risk figure for subsequent children in families in which a so-called sporadic case had occurred.

PLAN OF THE STUDY

Methods of securing patients' names. Several methods of attack were used to secure the names of children with the disease. First, the files of the Ohio Department of Vital Statistics for the years beginning January 1, 1940 and ending with December 31, 1955 were searched for *deaths caused* by retinoblastoma. Children born between Jan. 1, 1940 and Dec. 31, 1953 were chosen to derive an estimate of the mutation rate. Since a large percentage of children who develop the disease, do so within the first two years, those who died of it would be found by carrying the search for two years past the 1953 period. Those who did not die of it within that period, or did not die of it at all, would be found through the other methods of ascertainment.

Second, letters were sent to all the *hospitals* in Ohio that were not tuberculosis or mental institutions, requesting the names of all children who had been operated upon for retinoblastoma in the hospital from January 1, 1940 on, together with the names and addresses of the parents. Only those for whom the diagnosis had been based on pathological section were accepted.

Third, similar letters were sent to all the *physicians* in Ohio listed in the American Medical Directory as Ophthalmologists or Eye, Ear and Throat specialists, requesting the names and addresses of their patients who had been treated for the disease.

Fourth, *The State School for the Blind* in Columbus was asked for the names of the pupils who were there or had been there after 1940 and whose blindness was caused by bilateral retinoblastoma.

Finally, the *Ohio Services for the Blind* kindly let us have their records, and in this way we obtained those families who had applied for financial assistance for the treatment of retinoblastoma.

No one method would furnish all the names of the children with the disease

Received June 3, 1959.

who had been born in Ohio during the 14 year period under study. Those who had only unilateral disease would attend a regular school and so not occur on the lists of the School for the Blind. Those who had survived the disease would not be found in the search of the death certificates. Those who had been treated by physicians now deceased or moved from Ohio, or who had been sent to hospitals outside the state would not be found. Those who had not applied for financial assistance would not be obtained from the records of the Ohio Services for the Blind. From each source, therefore, names were obtained that had not been secured from other sources, but in many instances the same family was ascertained in several ways.

There were 152 letters sent to hospitals, and 75 were answered. The majority of those answering were the larger hospitals from which such cases would be expected to be drawn. Fifty-five of the hospitals responding to the questionnaire had no record of retinoblastoma cases for the years under study; 20 had one or more cases whose names and addresses were furnished. There were 357 letters sent to physicians. To both physicians and hospitals a personal and typed letter rather than a mimeographed one was sent, to insure the greatest possible individual attention from the recipient. Of these 357 physicians 191 had either died and their records had been destroyed, or they limited their practice entirely to Otology. Of the remaining 166 physicians, only 60 answered. Of these 29 had never had a case of retinoblastoma, and 31 forwarded the names and addresses of the parents of their patients.

This 36 per cent of those who might have had cases seemed a small return. It was felt, however, that many who did not answer neglected to do so because they had no cases to report, and did not realize that their failure to state this fact minimized the value of the study. This idea was supported, when in 1958, a second set of letters was sent to hospitals and to those who practiced Ophthalmology only, asking for all cases they had ever had if they had not responded to the first appeal, and for all those cases which they had treated before 1940, and after 1955 if they had answered the first letter. Many of the physicians and hospitals who had failed to respond the first time did so the second, either giving new names, or explaining that their lack of response to the first letter had been caused by the fact that they had never had a case of retinoblastoma in their practice. This brought the response of the physicians to about 80 per cent of those to whom the appeal was sent.

Ascertainment. It is certain that not all of the cases occurring in Ohio in those years were found. Nevertheless, the frequency with which a given case was obtained by more than one of the methods of ascertainment listed above gave some assurance that the list of patients secured was comprehensive. Table 1 shows the frequency of ascertainment of those cases which appeared to be sporadic, also of those which gave evidence of other members of the family being similarly affected. An attempt was made to determine how often any one proband *could* have been expected to be recorded in such a survey, and how often they actually were. In the hereditary cases, 9 of a total of 26, or 34.6 per cent were ascertained

TABLE 1. FREQUENCY OF ASCERTAINMENT OF HEREDITARY AND SPORADIC CASES OF RETINOBLASTOMA

	Hereditary Cases				Sporadic Cases				
Observed	1	2	3	4-8	1	2	3	4	5
Expected									
1	3								
2	5	10	2	1	56	15	6	2	
3	1	3	1		16	13	10		1

but once, while in the sporadic cases, 72 of 119 were ascertained but once, a percentage of 60.5. A test for similarity of the two groups from the standpoint of ascertainment gives a χ^2 value of 5.75, $P < .05$.

Although it was assumed that each proband could have been reported by at least one hospital and one physician, some probands were reported by several physicians, and in some instances by several hospitals, because the parents went from one to another in the hope of a better prognosis, or because the child had been operated upon for the condition in one eye in one hospital and for the other eye in another hospital. This makes for a greater number of ascertainments in the case of bilateral disease. In the case of one family, a proband was reported by four physicians, and by four hospitals, thus making 8 ascertainments when two would be expected. For example, a living child with unilateral disease, not applying for financial aid, would have two as the expected number of ascertainments. Death records, school records and Ohio Services for the Blind records would not be applicable. But the same child could have been seen by many physicians and in many hospitals all of which reported the case, thus making the actual number of ascertainments far exceed two. Since bilateral disease is more frequent among the hereditary cases, this helped to increase the number of ascertainments of hereditary retinoblastoma.

After the names and addresses of the parents of the probands were secured, a personal letter was written to each family, asking them to give (1) the names and birthdates of all the children born before and after the proband, (2) any of these children who had any eye trouble, (3) any relationship between the parents, (4) the eye which the tumor had affected if it were unilateral, and any other information that they desired to give. The birthplace of the child was also asked for, since those children born outside of Ohio could be used for some aspects of the study, but not for estimation of the mutation rate of the gene in Ohio. Later a field worker was sent to visit the family to obtain the extensive family history which could be got only by interview. The probands were in almost all instances small children so that the histories were obtained from their parents. Most of these parents were young, and many of their parents in turn were living, hence it was frequently possible to obtain accurate information on the great-grandparents of the probands and on all of their descendants. Because the dis-

ease occurs in the very young as a rule, (although there was one man whose onset was at about 61 years of age), the inquiry into the family history was carried until the last generation was reached. This sometimes involved going down at least two generations beyond the proband until information was forthcoming on all offspring of descendants of the great-grandparents.

All death records of deceased relatives were searched for whether the death occurred in Ohio or in other states, and all living relatives who had reached adulthood were written to for information on themselves, their brothers and sisters, their own children, grandchildren, etc. This involved a great deal of duplication, but it also insured against any relatives having retinoblastoma, and not being recorded. Although this method seemed to snowball into a family history without end, the insistence that the name of every relative, the age, the address, the names of their children and their grandchildren etc. be recorded, paid off in the end. It prevented inaccurate guesses on the part of the relatives, and it linked several families in which the retinoblastoma would have been regarded as sporadic had the usual method of asking merely for other affected relatives been adopted. These cases will be referred to later in the discussion of the individual families.

SPORADIC AND HEREDITARY CASES

The findings in this study were comparable to those in other studies of this disease, in that most of the retinoblastoma cases had no history of parents, sibs or collateral relatives being affected, even after an intensive investigation. Among the cases traced there were 26 probands who were hereditary cases, if by hereditary one designates all those instances in which one or more relatives were affected. There were 119 sporadic cases.

Among the names of probands sent in by physicians or hospitals, there were 19 who for one reason or another were not traced for complete family history. The record had stated in each instance that no family history of the condition was known. There were four cases in which the parents refused to co-operate. Three others were not located, because they had moved, and left no forwarding address. Twelve others were cases where the child had not been born in Ohio, but had come to a hospital in the state for treatment. Of these twelve, eight sent in partial reports, but their homes were too far removed for the field worker to call. The information given on these eight families was as follows.

1. Third of five children, died at 2½ years. Not known if uni- or bilateral.
2. First of two children, died at age 2.
3. First of three children, living. Second died at 2 months, no eye cancer.
4. Third of three children living at age 8 years: bilateral.
5. First of three children, living. Left eye at 4 years.
6. Sixth of seven children, living; unilateral. Last child is now aged 10.
7. First of two children, living, age 20 years; right eye.
8. Seventh of seven children, living at 6 years; left eye.
   The first child in this family has 2 normal children.
   The fourth child died at 1 year of pneumonia.

TABLE 2. LATERALITY OF RETINOBLASTOMA IN HEREDITARY AND SPORADIC CASES

Hereditary					Sporadic				
R	L	Bil.	Uni.	Unk.	R	L	Bil.	Uni.	Unk.
5	6	20*	3	3	35	39	37#	6	2

* 58.8 per cent of known cases bilateral. # 31.6% of known cases bilateral. $\chi^2_{(1)} = 8.38$ $P < .01$.

LATERALITY OF RETINOBLASTOMA

Of the 34 hereditary cases, including probands and their relatives, for whom it was known whether they were uni- or bilateral, 20 or 58.8 per cent were bilateral. Of the 117 sporadic cases, 37, or 31.6 per cent were bilateral. $\chi^2 = 8.38$, $P < .01$ (Table 2). All other workers have found that bilateral disease is much more common among the hereditary than among the sporadic cases. This has led to the belief that at least some of the sporadic cases are caused not by germinal but by somatic mutations, or that they are phenocopies. Of course, some of the unilateral cases may have died from the disease before they had a chance to exhibit their potentiality for developing the disease in the other eye. Or they may have been reported while still unilateral cases, and have become bilateral at some subsequent time. Thus the probands shown in Figures 7, 10 and 14, (V 7) were all unilateral cases at the time their names were reported, but had become bilateral by the time the interview was held, or shortly thereafter. Three of the unilateral cases which were hereditary, (Figures 4 and 5), died sufficiently soon following the operation on the first eye, that the subsequent fate of the second eye remains unknown. The longest time noted in this study between the ages of onset in the two eyes was 51 months. None of these three unilateral cases (Figures 4 and 5) survived this long. One such case, the proband, has lived over four years still unaffected in the second eye. Another unilateral hereditary case (Fig. 15, III-4) has lived for 43 years without the second eye being involved, although his brother and child were both bilateral cases.

In the unilateral cases in which the affected side was known, there were five right and six left in the hereditary series, and 35 right and 39 left in the sporadic group (Table 2).

Survival of Unilateral Versus Bilateral Cases

Although one bilateral case has lived about 51 years after his operation at the age of 2 years, and another has survived about 25 years past his operation at the age of 2, the percentage of bilaterally affected patients who succumb early to the disease is significantly greater than that among those who have but one eye involved. All probands and their affected relatives were listed as to whether they were sporadic or hereditary cases, whether they were uni- or bi-lateral cases, and whether they were dead or alive. Table 3 shows the results. When only one eye was involved about 20 per cent of the people were dead, whether they were sporadic or hereditary cases. When both eyes were affected, 37.8 per cent of the sporadic cases and 50 per cent of the hereditary cases had died. Bilateral cases, therefore, have a much diminished chance of survival, $\chi^2_{(1)} = 8.42$,

TABLE 3. PROBABILITY OF SURVIVAL IN UNILATERAL AND BILATERAL RETINOBLASTOMA

Sporadic				Hereditary			
Unilateral		Bilateral		Unilateral		Bilateral	
Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
16	64	14	23	3	11	10	10
20.0%		37.8%		21.4%		50.0%	

P < .01. Although it would appear that the hereditary bilateral cases have a greater risk of dying than the sporadic bilateral cases, the difference between them is not significant. $\chi^2_{(1)} = .81$, P > .30.

PARENTAL AGE

Although one would not expect that parental age would influence the appearance of retinoblastoma of the hereditary type, since the mutation causing these cases always had to occur in a generation before that of the parents of the affected children, it might be thought that parental age could influence the development of unilateral sporadic cases which might be caused by somatic or germinal mutations or which might be phenocopies. The bilateral sporadic cases, which are apparently caused by germinal mutation or are phenocopies, might likewise be affected by parental age. It is conceivable that bilateral cases might be caused by somatic mutations, but it is highly improbable that two identical mutations occur in the two eyes of the child. If the mutation is conceived of as having occurred past the zygote stage but before the embryo has had its bilaterality laid down, the period of occurrence would be very limited. It seems more credible to assume that bilateral cases are not somatic mutations. The cases of retinoblastoma were divided, therefore, into three categories, unilateral sporadic, bilateral sporadic, and hereditary cases, whether uni- or bilateral. The ages of the parents at the birth of their normal and affected children were computed in years and months. The difference in the average ages of mothers at the birth of their normal children and unilaterally or bilaterally affected sporadic cases was much less than the standard error of the difference. There was no significant difference in the average ages when mothers of unilateral and bilateral sporadic cases were compared, or when either of these were compared with the average age of mothers of children with the hereditary type, although the latter was higher than the average age of mothers of the sporadic cases.

When the father's ages were compared, however, some difference did occur. There was no significant difference in the average age of the fathers of unilateral as compared with bilateral sporadic cases, or with the average age of these fathers when they had their normal children but the age of fathers of hereditary cases was much higher than that of fathers of sporadic cases (t = 3.7, P < .001). This significant difference arose entirely because of one family, in which there were six sibs affected bilaterally, and in which the father was 18 years and 5 months older than the mother. This large difference was entered for each of the six affected sibs as well as for the normal sibs, thus accounting for all of

the excess in the average age of the fathers of hereditary cases as contrasted with the average age of the fathers of sporadic cases. It seems reasonable to conclude that in this series there is no association between parental age and the appearance of the disease.

Falls and Neel (1951) found no significant difference between the parental ages of sporadic and hereditary cases, although the former tended to be higher than the latter. The reverse of this was found in this study.

Place in Family

If parental age shows no effect, it is reasonable to conclude that place in family probably will show no effect. This proved to be the case when the Greenwood-Yule method of determining expected place in the family was used. This agrees with the findings of Falls and Neel.

Consanguinity of Parents

Two of the 145 matings (1.4 per cent) were between related parents. Both instances occurred in the same pedigree (Figures 4 and 5); both resulted in children with retinoblastoma. Many marriages between close relatives occurred in this family, which lived in a small, isolated section of Kentucky. Most of these consanguineous matings did not produce children with retinoblastoma. It is believed, therefore, that the association between the occurrence of the disease and the consanguinity in the parents is fortuitous. One of the marriages producing an affected child was not between related parents. The wife came from a different part of the country. The wife might have also carried the gene, and the whole pedigree could then be explained on the basis of the disease being caused by a recessive gene in this family. The infrequent occurrence of consanguineous matings in other series of cases reported in the literature would lead one to reject that explanation in this family, and to interpret the pedigree as exhibiting a dominant gene with low penetrance.

FREQUENCY IN THE POPULATION

All children who were not born in Ohio in the 17 years under study were excluded in making this estimate. There were 126 children found in this study who were born in Ohio between January 1, 1940 and December 31, 1956, who developed retinoblastoma. Excluding those dying under one year, there were 2,934,247 children born in Ohio during those 17 years. Thus one in every 23,287 children developed the disease. In round figures, it might be stated that 1 in every 25,000 children who survive to the age of one will be likely to develop retinoblastoma in Ohio, if this figure prevails over the coming years.

EMPIRIC RISK FOR FUTURE CHILDREN

This is a matter of some disagreement among workers. Reese (1946) formerly stated that if an adult is affected he advises the patient not to have children because the risk of producing affected children is too great, but that if normal parents have a child with retinoblastoma, he has no hesitancy in advising them

to have more children. In a conversation with Dr. Reese in 1958, he stated that he had materially changed that attitude because he had seen several cases in which normal parents with one affected child had produced other affected offspring.

The question of risk of future affected children has several aspects. One is the question asked by the normal parents who have produced one child with retinoblastoma as to the probability of their future children being affected. The second is the question asked by the affected persons themselves when the matter of their producing children arises. For many, the second question does not arise; the patient has died before the age of reproduction has been reached.

After the first group of cases born between January 1940 and the end of 1953 was investigated, a second set of letters requesting cases before and after those dates was mailed. This was to find persons, from those born before 1940, who had the disease and who had lived and had had children, to determine the number of affected offspring. The response for the names of patients occurring before 1940 was meagre, and of the names furnished, it was almost impossible to trace many of the families from the addresses obtained at that time. Fifteen families whose affected children were born between 1932 and 1940 were traced, however. Only three sporadic cases had married and had children. Two were cooperative and gave their history. They had one normal child each. The third patient may have been cooperative had we been able to obtain her married name and address, but her father, a physician, was adamant in refusing to give us any information about her, her whereabouts, or her children.

Two other families in which parents were affected were traced. This makes a total of four families with two having affected children. Before one can say that the two parents whose offspring are normal are sporadic cases caused by somatic mutations or are phenocopies, one must wait until their families have been completed, and until the children have had a reasonable length of time to develop the disease. If any of their offspring do become affected, one knows that they were hereditary cases. If none become affected, the matter still is unanswered since the parents might belong to families in which the penetrance was low.

Another family in which the person was reported as having unilateral retinoblastoma was so unusual that it was not included. The patient was a man, whose eye was removed at the age of 67 for the disease. Most of his relatives were in Europe, but his four sibs and their children were normal, as were his own four grown children, grandchildren and small great grandchildren.

Several studies have been made on the problem of the frequency of the disease in the offspring of persons who appear to be sporadic cases of the disease. Tucker, Steinberg and Cogan (1957) have recently reviewed the work done on this, and have reported 8 additional families in which a unilaterally affected sporadic case has had children. Two parents had two affected out of three children, the other six had twelve unaffected children past the age of 3, and three others under that age. These authors conclude that on the basis of the scanty data available approximately 25 per cent of the sporadic cases are likely to

transmit the disease to their children, and that when they do, 40 to 50 per cent of their offspring may be affected. They quote Vogel as stating that only 25 per cent of sporadic cases represent mutations, (this author presumes that germinal mutations are meant), and their findings are in agreement.

These authors quote Hemmes as having found four instances in which a person with unilateral involvement had married and produced nine children of whom seven were past the age at which the disease might be expected to develop. All nine were normal. They cite the pedigree of Lange in which a unilaterally affected parent had one of four children affected. These cases indicate the relatively low rate of transmission by sporadic cases.

The request made by Tucker and his co-workers that many centers collect data and publish them in detail is well made. However, unless some identification of the families is possible, the same unusual families may be replicated thus distorting the picture. Although Family 115 of this study is not an instance in which a parent is a sporadic case, it is unusual in that three of five children were affected. This family is being reported for the first time. The children were seen by physicians in Ohio and Michigan who might well choose to report it. A collection of cases from the literature in the future would show three families each with three children affected when there was but one.

It may be that from an accurate compilation of data some more comprehensive understanding of the inheritance of retinoblastoma will emerge, and that a more intelligent answer will be forthcoming to questions of normal parents who have had one child affected, and of affected persons who appear to be sporadic cases, as to future risk. It should be pointed out that, if the degree of penetrance may fall as low as 20 to 30 per cent as seems to have occurred in some of the families in this study, the estimate of Vogel's that only 25 per cent of sporadic cases are mutations will have to be revised. The low degree of penetrance may be responsible for transmission by only 25 per cent of the sporadic cases, who might after all, be largely or entirely caused by mutations.

With respect to the problem of future risk when normal parents have produced one affected child, some information can be gained from the present study. There are 145 sibships in these families in which the parents were normal. In 57 of them, the affected offspring was either the only child or was the last in the family. The fact that some normal children have preceded the affected child gives some assurance that the parents may not have been carrying the gene nonpenetrant. If the family is large, this assurance has more validity; if the family is small, it may mean little. Family 14 (Fig. 7) is a case in point. The mother had had 16 normal children by two husbands before her third marriage. If the gene is a dominant, this meant that there was only one chance in 65,536 that she had the gene and had failed to pass it on. Hence, even with a low degree of penetrance, it was unlikely that she was carrying the gene without the disease showing in some of her children.

There are 88 sibships remaining whose parents were normal. In 6, or 6.8 per cent, the first affected child had been followed by other affected children. If one considers the number of children born after the first affected child in all

the families listed here in which the parents were normal, there were 208 children of whom 14 developed retinoblastoma, or 6.7 per cent. In this series whether one counts sibships or children in whom this disease will be repeated, when the parents are normal, the probabilities are the same, namely, that about one in 15 will develop retinoblastoma after one child has shown the condition.

DESCRIPTION OF PEDIGREES

Penetrance and the mutation rate will be considered after the discussion of the pedigrees. The extent to which the investigation was carried in each of the families is indicated in Table 4. No one was included in this table unless accurate information had been obtained from the person himself, or from his parents, his children or his sibs. If the number of relatives were known, but they could not be traced, they are included with a question mark after them to show that although they were said not to be blind by the proband's family, no verification of this had been possible through direct contact. The anonymity of the families has been preserved, but future investigators of retinoblastoma in Ohio can determine whether their patients have come from one of these probands or his sibs. In Table 4, where the families are listed, an indication of the family surname is furnished by giving the total number of letters in the name followed by the first two letters. The sex, initial, and birthdate of each sib is shown, and the proband is indicated by italic type. For example, if the surname is Black, the family is designated as 5 Bl. If John is the proband and survives, his children, if any, can be studied 25 years hence, and the family history will be known as it exists now. If he has a sister Sheila, who marries a man named Jones, and if they have a child with retinoblastoma, inquiry into her maiden name, her birthdate and names of her sibs, will reveal that she belongs to this family whose history is already known.

The families with more than one affected person are shown in Figures 1–15. They are drawn in skeleton outline only, to reveal the relationships between the affected persons, which are often obscured when all relatives investigated are filled in. When it is obvious through which parent the mutant gene is transmitted, the other parent and all relatives may be omitted. Thus the number of sibs of grandparents, and their descendants shown in the pedigrees may not correspond with the number given in Table 4. In Figure 6, for example (Families 55 and 62) the pedigree shows only two sibs (III-1 & 3), sibs of the grandparents of V-3, but Table 4 lists five. This is because the wife of III-2 and her sibs are not shown. Sometimes the first generation given in the pedigree is not listed in Table 4, but is the last generation in which the mutation could have arisen to have it present in the two or more collateral lines exhibiting the trait.

Family 77 (Figure 1) shows two of three children affected. The father (II-1) was an adopted child who knew nothing of his family. The first child had both eyes removed and died at the age of 19 months. The parents had been assured by the surgeon performing the operation that there was no risk of a second child developing the condition since they were normal. Unfortunately this opti-

mistic attitude was not justified because the second child developed bilateral retinoblastoma also. The left eye was enucleated, but the second received diathermy and TEM treatment.

Family 115 is shown in Figure 2. The entire family tree is presented since it is not known through which parent the gene is transmitted. The disease was bilateral in the brother, unilateral at present in the next child, and unilateral in the third child, born in February 1959, at the age of 3 months.

Family 12 (Figure 3) is the same family as that reported by Sowik (1952). Much more information was secured on the family than is listed in Fig. 3. All six children were probands, ascertained independently, and through several channels. All were bilateral, three have died of the disease. The mother had a small, undeveloped eye in childhood with little vision on that side. According to the view expressed by some ophthalmologists, this might have been a rare example of spontaneously regressing retinoblastoma. The mother refuses to have this eye examined. Sowik stated that the father met any strangers at the door with a shotgun, so the family history was obtained from a brother of the father, and a sister of the mother. Later, the field worker secured an interview with the father.

Family 60 combines the features of sibs as well as collateral relatives being affected. The first contact with the family came through the proband V-5, in Figure 4. The interview revealed that three older sisters had died with the disease. This family came from Kentucky. The death certificates of the three sisters were obtained, and all gave retinoblastoma as the cause of death. The father and mother were related, but they were not sure how, and this was not known until the family was reinvestigated following the finding that a new proband (VI-1) belonged to this family also. IV-2 had been raised by his maternal grandfather after his mother had died in childbirth. He used his grandfather's name rather than his own. He knew that two brothers and a sister of his mother had died at the age of 2 years with the same eye cancer that had afflicted four of his children. He knew of no other instances in the family. The death certificates of his aunt and uncles (III-3, 4 and 5) were found and the cause of death was given as "glioma of the retina", the name formerly applied to retinoblastoma.

While the field worker was in the area in Kentucky in which this family had originated, information was secured on a child that had died with bilateral retinoblastoma, and the maiden name of the mother was the same as the real surname of the father of the four affected children who had been first investigated. The mother denied any relationship to the group of persons shown on the left side of Figure 4. The death certificate of her child was found and the cause of death was given as retinoblastoma.

Later, another proband was found in northern Ohio (Family 63), and many of the names present in his paternal line were also found in the relatives in Family 60. A return trip to Kentucky clarified the situation. Since many persons in this family marry very young, it was possible to interview some of the sisters of II-3, and to establish the relationships between IV-5, VI-1 and the other seven affected persons in Figure 4. This more intensive investigation revealed

TABLE 4. SUMMARY OF INVESTIGATED RELATIVES OF PROBANDS WITH RETINOBLASTOMA*

Fam. No.	Family Name		G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des. P. S. C.	Sibship	Sibs'C
1	5 AD	P	4	2	10	19, 37, 11, 2	+	5	5	9	♂ H. 3/3/41. ♂ *R.* 4/41/45. Rt. at 20 mos. ♂ L. 11/21/53	
		M	4	2	1	1, 2	+	3	5			
2	8 AN	P	4	2	13	48, 20	+	1			♂ R. 7/3/51. ½ bro. by mo. ♂ *D.* 6/15/55. Onset 3 mos. Bilateral at 16 mos.	
		M	4	2	15	82, 101, 5	+	5	4			
3	7 Ba	P	4	2	10	27, 43, 29	+	3	5	5	♂ *L.* 12/18/52; d. 3/12/55. Rt. at 9 mos. ♀ R. 12/21/55	
		M	4	2	13	44, 67, 3	+	6	4			
4	7 Ba	P	4	2	4	16?	+	5	17	5	♀ *Mrs. A.B.* 8/10/37. Le. at 12 mos. Has one son L. 5/1/56. ♂ S.J. 5/9/1/41. ♂ R.J. 8/3/43. . . B.J. 6/1/45. ♂ T.J. 7/20/49. ♀ C.J. 4/11/52	
		M	4	2	12	34, 39, 24, 6	+	5	11	3		
5	6 Ba	P	4	2	2	4, 2	+	5	5	1	♀ D. 3/15/40. ♂ P. 12/19/46. ♂ *C.* 3/31/52. Bilateral. Rt. at 25 mos. Le. at 46 mos.	
		M	4	2	13	35, 61, 16	+	3	6			
6	7 Ba	P	4	2	20	70, 46	+	7	14	2	♀ V. 8/27/53. ♀ *K.* 9/14/56. Rt. at 17 most. ♀ K. 6/14/58	
		M	4	2	10	12, 23	+	6	12	4		
7	9 Be	P	4	2	1	2, 5	+	1	2		♀ *G.* 3/8/43. Le. at 3 yrs.	
		M	4	2	9	19, 24, 1	+	1				
8	6 Bi	P	4	2	9	17, 15	+	3	2		♂ M. 2/12/48. ♀ *D.* 5/2/50. Le. at 9 mos. Rt. at 5 yrs. ♂ R. 2/5/53	
		M	4	2	18	37, 14	+	6	4			
9	7 Br	P	4	2	10	24, 49, 63, 3	+	4	8	7	♂ *T.* 7/14/47. Le. at 2 yrs. d. at 4 yrs. ♂ J.A. 9/18/49. ♀ P. 7/1/50. ♀ J. 3/20/55	
		M	4	2	8 3?	22, 18	+	10	20			

10	6 Br	P	No information				+	1	2	8	♂ *S*. 7/23/37. Le. at 2 yrs.	
		M	4	2	7	5, 1	+	6	9			
11	5 Bu	P	4	2	12?	?	+	6	13	1	♂ R. 4/27/39. ♂ *D*. 7/?/41, d. 1/44. Rt. at 18 mos. ♀ M.A. 7/25/54.	
		M	4	2	8	28, 93, 43	+	3	18			
12	5 Ca	P	4	2	12	17 (1?), 35 (9?), 26	+	4	6		See Fig. 3 only skeleton pedigree in figure.	
		M	4	2	8 (4?)	12 (8?), 6	+	9	21		♂ *S*. 9/22/29. d. at 39 mos. Bilateral. ♂ *G*. 9/30/31. Bilateral. ♂ C. 7/16/33. ♀ B. 9/28/35. ♀ *G*. 10/19/37. d. 53 mos. Bilateral. ♂ *R*. 6/2/40. d. at 35 mos. Bilateral. ♀ M. 8/6/43. ♂ *E*. 11/8/46. Bilateral. ♂ *J*. 10/24/48. Bilateral. ♀ L. 3/27/49	
13	7 Ca	P	4	2	?		+	3	8		♂ *D*. 8/12/41. Rt. at 18 mos.	
		M	4	2	?		+	7	11			
14	9 Ca	P	4	2	12	37, 85, 23	+	9	20		See Fig. 7.	
		M	4	2	6	14, 33	+	7	21		♂ W. ½ bro. by mo. 6/21/30. d. at 16 mos. ½ sibs by mo.'s 2nd m. ♀ Mrs. D.M. 5/11/32. ♀ A.W. 11/19/33. ♂ J.W. 9/10/35. ♀ E.J.W. 7/13/37. ♂ C.W. 4/9/40. J.W. and G.W. Id. ♂ twins. 5/29/41. d. at 11 mos. ♂♀ twins K.W. and K.W. 6/3/42. ♂ d. at 11 yrs. ♀ E.W. 11/9/44. ♀ A.W. 1/17/45. ♂ J.W. 8/13/46. ♀ L.W. 1/13/48. ♀ K.W. 2/5/49. ♀ S.W. 3/11/50. Full sib. by 3rd. m. of mo. ♂ R.C. 12/4/52. ♂ *D.C.* 1/?/54. Eyes removed at 10 and 20 mos.	2

* See page 29 for explanations of abbreviations.

TABLE 4—*continued*

Fam. No.	Family Name		G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des, P. S. C.	Sibship	Sibs'C
15	12 Co	P	?	2	8?		+	6	5	3	♂ B. 6/16/47. ♀ *L.* 5/29/49. d. 34 mos. Rt. eye at birth, Le. at 1 yr. ♂ M. 9/21/51. ♂ T. 8/12/55	
		M	4	2	7 (2?)	13, 14	+	3	9			
16	3 Co	P	4	2	7	13, 19, 5	+	3	3		♀ M. 10/6/45. ♂ W. 6/26/47. ♀ J. 7/31/48. ♂ *R.* 4/20/51. Rt. at 43 mos.	
		M	4	2	10	28, 46	+	2	6	1		
17	6 Co	P	4	2	12	30, 42 (4?)	+	11	9		♂ *T.* 7/20/47. Bilateral at 19 mos. Noted at 2 mos. ♂ D. 12/27/48. 2 st. b.	
		M	4	2	4	8, 9	+	1	2			
18	7 Co	P	4	2	12	28, 51, 24	+				♂ N. 3/17/20. ♀ Mrs. M.G. 5/20/16. ♀ Mrs. D.W. 10/15/18. ♀ Mrs. T.W. 9/1/21. ♂ *G.* 1/7/34. Le. at 2 yrs. d. 25 mos.	11
		M	4	2	7	23, 2	+	8	27	38		
19	5 Cr	P	4	2	14	65, 13	+	5	3		♂ J. 11/28/53. ♀ V. 2/27/55. ♂ *M.* 1/10/57. d. at 20 mos. Bilateral, at 1 mo.	
		M	4	2	9	15, 4	+	10	5			
20	5 Cr Negro	P	No information				+	No information			♀ L. 6/9/45. of ♂ J. 6/4/47. By mo. 1st m. Full sibs by mo. 2nd. m. ♀ *C.* 5/18/50. Le. at 5 yrs. ♀ ♀ G. and G. 7/19/54. ♀ L. 9/13/55. ♀ P. 9/6/56. ♂ J. 12/2/57	
		M	4	2	15 (7?)	19?, 11, 9	+	4	17			
21	6 Cu	P	4	2	13	40, 98, 75	+	12	50	6	♂ *J.* 5/18/47. Rt. at 9 mos. ♀ C. 12/29/56	
		M	4	2	9	4, 10	+	6	7			

22	7 Da	P	4	2	1	4, 5	+	3	10		♀ *S*. 11/?/47. Unil. at 2 yrs. d. 42 mos. ♀ E. 1/30/55
		M	4	2	13	35, 54, 9	+	6	8	3	
23	4 De See Fig. 13	P	4	2	10	16, 43, 15	+	5	8	2	♀ M. 1/27/47. ♂ *C*. 2/7/50. Rt. at 27 mos.
		M	4	2	3	12, 22	+	2	2		
24	9 De	P	4	2	6	7, 8	+	1	2		♀ P. 1943. ♀ *K*. 1946. d. at 5 yrs. Le. at 2 yrs. ♂ R. 1953
		M	2	2	?		+	7	13	10	
25	3 Dy	P	4	2	5	8, 6	+	4	3		♀ S. 2/19/47. ♀ *C.J*. 1/27/48. Le. at 6 yrs. ♂ T. 7/25/49. ♀ J. 7/16/50. ♀ E. 9/27/51
		M	4	2	9	25, 19	+	3	6		
26	8 Eb	P	4	2	13	44, 62, 9	+	5	11		½ sib ♂ C. 4/14/41. ♂ *W*. 10/11/51. Le. at 2 mos.
		M	4	2	9	30, 31	+	4	14		
27	10 Ec	P	4	2	14	33, 65	+	7	26	2	♀ C. 9/29/46. ♀ M. 1/4/48. ♂ W. 7/26/54. ♂ *J*. 7/26/55. Le. at 19 mos.
		M	4	2	8	13, 18	+	4	7		
28	5 El	P	4	2	9	36, 80, 34	+	3	9		♀ D. 4/20/41. ♂ K. 3/25/42. ♂ R. 11/22/43. ♀ *J*. 11/7/46. Rt. at 56 mos. ♂ S. 1/13/51
		M	4	2	10	12, 18	+				
29	5 Ev	P	4	2	10	14, 26, 8	+	1	2		♂ J. 4/28/41. ♀ *P*. 6/2/44. Bilateral at 3 yrs. d. 6 yrs. 8 mos.
		M	4	2	8	9, 7, 3	+	3	3	1	
30	5 Ev	P	4	2	12	25, 56, 17	+	6	5		♂ M. 2/14/44. ♂ *T*. 9/18/46. Le. at 51 mos.
		M	4	2	14	27, 31, 2	+	3	3		
31	8 Fe Negro	P	4	2	11 (10?)	4, 8	+	5	10		♀ *A*. 10/30/52. Le. at 26 mos. ♂ *D*. 1/11/54
		M	4	2	9	?	+	3	2		

TABLE 4—*continued*

Fam. No.	Family Name		G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des. P. S. C.	Sibship	Sibs'C
32	8 Fe	P	4	2	14	19, 2	+	7	8	1	♀ M. 8/17/37 ♂ M. 8/17/39. ½ sibs by father. Full sibs. ♀ C. 7/29/55. ♂ *R.* 12/21/56. Rt. at 4 wks.	
		M	4	2	10	17, 4	+	6	10			
33	6 Fi Negro	P	4	2	12	13, 25	+	5	14		♂ W. 9/22/33. ♂ R. 8/3/38. ♀ J. 9/22/42. ♂ *C.* 11/19/44. Rt. at 4 ½ yrs.	1
		M	4*	2	13 (10?)	12, ?	+	16	?			
34	8 Fo	P	4	2	5	1, 3	+	4			♀ R. 1/9/48. ♂ J. 12/26/48. ♂ M. 11/6/50. ♂ M. 11/26/52. ♂ C. 5/16/54. ♂ *R.* 5/26/55. Le. at 23 mos. ♂ T. 11/7/57	
		M	4	2	4	6	+	5	3			
35	8 Fr	P	4	2	11	8, 12, more?	+	1	2		♂ *T.* 12/2/41. Bilateral at 5 mos. ♂ R. 2/9/45. ♂ R. 5/9/47	
		M	?	2	14	29?	+	4	10			
36	3 Ga	P	No information				+	No information			♂ *A.* 1/13/48. d. 3 yrs. 11 mos. Bilateral	
		M	No information				+	No information				
37	3 Ge	P	4	2	6†	24, 51, 39	+	9	11		♂ A. 4/5/33. ♂ G. 12/7/34. ♂ B. 7/29/36. ♂ G. 7/26/39. ♂ D. 3/11/45. ♂ *B.* 2/4/51. Rt. at 18 mos.	3
		M	4	2	11	39 (18?), 44, 10	+	9	16	3, 3		
38	6 Ge	P	?	2	7 (2?)	48, 10	+	8	18		♀ S. 12/14/38. ♂ J. 7/25/40. ♀ *M.* 12/22/41. Rt. at 18 mos.	
		M	?	2	?	?	+					
39	5 Ge	P	4	2	5	5, 2	+	3	3		♂ *D.* 8/16/41. d. 7 yrs. Bilateral at 5 yrs. ♂ A. 6/?/44. ♂ L. 1/?/48	
		M	4	2	?	1, 1, 5, 2	+	4	6	2		
40	5 Go See Fig. 11	P	4	2	16‡	19, 40, 45	+	1	1		♀ C. d. at 4 days. ♀ Mrs. M.H. 11/28/30. ♀ *J.* 11/17/36. d. 4 yrs. Le. at 21 mos. Rt. at 33 mos.	3
		M	4	2	7	39, 50, 90, 42	+	2	3	13§		

41	6 Go	P	4	2	15	41‖, 74, 25	+	5	4		♂♂ J. and A. 9/7/50. ♂ *D.* 4/8/52. One eye at 8 mos. 2nd. at 3 yrs.	
	See Fig. 15	M	4	2	11	24, 47, 14	+	8	23	14		
42	8 Gr	P	4	2	13	27, 66, 55	+	2			♂ D. 1/21/47. ♀ *L.* 8/10/49. Le. 13 mos. Rt. X-ray. ♀ D. 7/27/56	
		M	4	2	11	22, 24, 6	+	4	7			
43	5 Gr	P	4	2	9	16, 16	+				♀ D. 9/7/49. ♂ *G.* 4/30/53. Le. at 3 yrs. 8 mos.	
		M	4	2	3	7, 12	+	1	2			
44	6 Ha	P	4	2	8 (3?)	3	+				½ sibs by father. ♂ J. 5/28/47. ♂ J. 12/27/49. ♀ D. 9/28/51. Full sibs. ♂ *E.* 10/7/52. Rt. at 9 mos. ♂ J. 5/28/54. ♀ N. 10/4/55	
		M	4 (2?)	2	1	1	+	1				
45	6 He	P	4	2	8	20, 33, 51, 6	+	2	3	9, 6	½ sib by father. ♂ J. 2/10/21. Full sibs. ♂ G. 5/27/34. ♂ *J.* 3/1/49. Onset 4 mos. Unil.	
		M	4	2	6	8, 6, 6¶	+	4	6	3		
46	6 Ho	P	4	2	7	17, 31, 41	+	4	12	17	♀ Mrs. J.B. 9131. No. ch. ♂ J. 1932. (1 ch.). ♂ G. 1934. ♂ D. 1935. ♀ R. 1936. ♀ M. 8/20/41. ♀ C. 12/25/42. ♂ R. 4/20/44. ♂ J. 6/28/45. ♀ L. 6/18/47. ♀ *A.* 12/1/48. Le. at 9 mos. ♂ K. 3/31/50. Drowned at 17 mos. ♀ K. 3/31/51	1
		M	4	2	7	5, 10	+	6	18	2		

* One had eye removed, age and cause unknown.
† One born with undeveloped eye; same condition present in his children and grandchildren.
‡ ♂ T. d. at 3 yrs. 10 mos. ca. of eye, metastases to skull.
§ One d. of Wilm's tumor.
‖ One said to have died of retinoblastoma at 2 yrs.
¶ 2 in a sibship of 3 have primary optic atrophy and mental retardation.

TABLE 4—*continued*

Fam. No.	Family Name		G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des, P. S. C.	Sibship	Sibs'C
47	7 Jo See Fig. 14	P	4 (2?)	2	12 (3?)	16 (8?), 6	+	Rt. 3* at 2 yrs.	6	?	♂ D. 10/7/38. (1 ch.). ♀ C. 10/17/43. ♀ J. 9/23/46. ♀ *J*. 10/14/50. Rt. at 8 mos. Le. at 6 yrs.	1 ♀
		M	4	2	5	8	+	4	14			
48	5 Jo Negro	P	?	2	15?	?	+	2	2		♀ *L*. 5/13/52. Le. at 18 mos. ♀ A. 12/22/53. ♂ L. 3/55	
		M	?	2	2?	?	+	6	14			
49	6 Jo	P	4	2	11	16	+	9	19	7	♂ *R*. 9/3/47. Le. 16 mos. ♀ L. 8/11/54	
		M	4	2	4	8	+	1	2			
50	8 Ju	P	4	2	7	9, 11, 5	+	1	2		♂ *D*. Le. at 1 yr. ♂ R. 4/16/57	
		M	4	2	12	17, 32, 6	+	2	4			
51	6 Ka	P	4	2	7	7, 10	+	2	4		♀ *A*. 3/5/55. Rt. at 18 mos.	
		M	4	2	11	41 (12?), 12	+	2	3			
52	5 Ke See Fig. 9	P	4	2	11	30, 41†	+	6	11		♂ *P*. 2/20/48. Eye rem. age 2. ♂ P. 5/2/51	
		M	4	2	4	7	+	1	3			
53	9 Kl	P	?	?	1 (others?)	4	+	4	6		♀ S. 6/29/44. ♀ S. 8/6/47. ♂ *J*. 8/13/49. Le. at 15 mos. of J. 8/16/50. ♂ F. 12/5/52. ♂ R. 12/4/55	
		M	4	2	5	8, 16	+	4	9			
54	4 Ko	P	4	2	9	11, 7	+	1			♀ K. 1/2/43. ♂ R. 12/1/44. ♀ *M*. 6/3/48. d age 6 yrs 10 mos. Rt. at 6 yrs. ♂ H. 5/22/51. ♂ T. 10/25/52. ♂ D. 2/27/56	
		M	4	2	2	7, 8, 1	+	10	12			

55	4 Ko See Fig. 6	P	4	2	11	18, 19, 1	+	10	26	3+	♂T. 3/19/42. ♂ *D*. 3/3/47. Le. at 5 ½ yrs. ♂ G. 10/12/48. ♀ D. 12/?/52
		M	4	2	6	6, 3	+	3	3		
56	6 Ko	P	4	2	6	6	+	3	4		♂ *D*. 10/29/55. Bilateral. Rt. at birth, Le. at 9 mos. ♀ K. 5/2/57
		M	4	2	17	62, 43	+	2	1		
57	6 Kr	P	?	2 (1?)	?		+	8	15	6	♂ *R*. 3/14/45. Rt at 5 mos. ♀ S. 7/4/48
		M	?	2	1	2?	+	1	1		
58	8 LaC	P	4	2	7	25, 45	+	7	14	4	♂ D. 6/17/49. ♀ *D*. 1/18/56. Bilateral at 19 mos.
		M	4	2	5	13, 10	+	1			
59	7 Le	P	4	2	6	9, 14, 4	+	4	6	1	♀ *J*. 6/10/39. d at 5 ½ yrs. Le. onset ? ♀ B. st. b. ♂ G. 8/27/47
		M	4	2	4	10, 12, 2	+	4	9	6	
60	6 Lu See Figs. 4 and 5	P	4	2	15‡	50, 88	+				♀ *R*. 10/21/37. d. 2 yrs. Bilateral onset at 15 mos. ♀ D. 7/19/40 d. at 2 yrs. ♀ *H*. 11/12/42. d. 2 ½ yrs. Le. eye. ♂ *K*. 3/29/46. d. 22 mos. Rt. eye. ♀ K. 6/4/47. ♂ F. 5/15/49. ♂ C. 11/8/50. ♂ *G*. 2/10/53. Rt. at 18 mos. ♂ ? /12/?/54
		M	4	2	18	126, 374, 13	+	5	27		
			Over 4,000 relatives of this family have been traced. Only a skeleton pedigree is shown in Figs. 4 and 5.								
61	6 Ly	P	4	2	8	20, 1	+	4	9		♂ J. 2/6/36. ♂ R. 7/27/42. ♂ J. 3/3/48. ♂ *T*. 10/2/52. Rt. at 2 mos. Le. at 22 mos.
		M	4	2	7	20, 16	+	14§	2		

* Bro. of father bilateral, d. age 4 yrs.
† One had unil. retinoblastoma.
‡ 3 died with glioma retinae at 2 yrs.
§ Children of 12 lost track of.

MVM

TABLE 4—*continued*

Fam. No.	Family Name	G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des. P. S. C.	Sibship	Sibs'C
62	6 Ma P See Fig. 6 V3	4 (2?)	2	5	19 (7?), 27, 45*, 3	+	9	7	8	♂ H. 6/1/35. ♀ Mrs. F.L. 8/15/36. ♂ L. st. b. ♀ E. 4/10/40. ♂ R. 4/1/41. ♂ A. d. 25 days. ♂ *L.* 7/27/44. d. at 8 yrs. Bilateral at 2 yrs. ♀ I. 12/20/49. ♂ T. 12/29/51	
	M	4	2	21	46 (4?), 42 some ?	+	6 full 2 half 5?	25			
63	7 Ma P See Figs. 4 and 5	4	2	21	78, 10	+	7	4		♂ *S.* 3/8/55. d. 26 mos. Le. at 2 yrs. ♂ R. 3/7/56	
	M	4	2	15	31, 17, 1	+	4	5			
64	6 Ma P	4	2	7	23, 13	+	8	20	23	♀ M. 1914, d. 1928. ♀ R. 6/12/17 d. 1943. ♀ H. 10/3/18. d. 5 mos. of 2/17/21. ♂ C. 11/17/23. ♂ G. 1/30/26. ♀ Mrs. M.S. 2/17/31. ♀ *L.* Mrs. V.G. 1928. ♂ H. 7/12/31. ♀ *L.* 7/16/36. d. 39 mos. Unil. at 29 mos. ♀ Mrs. D.S. 2/21/37	2 4, 4 2
	M	4	2	19	30, 43, 4	+	6	11	1		
65	7 Me P See Fig. 12	4	2	22	8, 10, 2	+	3	10		♂ R. 4/11/37. ♂ *T.* 8/10/40. Rt. at 2 yrs. ♂ J. 4/15/43. ♀ J. 6/9/44. ♂ J. 6/25/46. ♂ T. 6/20/48. ♂ C. 4/12/52	
	M	4	2	21	56, 112, 126	+	8	11	2		
66	5 Mi P See Fi g. 10 10	4	2	9	13, 7	+	1	1		♀ *S.* 3/6/55. Le. at 30 mos.	
	M	4	2	5	12, 3	+	6	4†			
67	5 Mi P	?	2	?	?	+	8	24		♂ *J.* 8/22/45. ♂ E. 10/31/48. ♂ *T.* 8/15/52. Rt. at 27 mos.	
	M	4	2	20	40, 6	+	4	8			

* See Family 55.
† A 1st cousin of the proband also had retinoblastoma.

† A 1st cousin of the proband also had retinoblastoma.

68	5 Mi	P	4 (2?)	2	3 (some?)	10, 9	+	4	11		♂ R. 9/27/46. ♀ J. 10/30/47. ♂ T. 8/29/50. ♀ A. 6/23/53. ♀ J. 7/9/55 ♂ *W*. 9/23/56. Bilateral at 15 mos.	
		M	?	2	6	14, 11, 4	+	8	4			
69	7 My	P	4	2	7	9, 13	+	3	4	12	♀ Mrs. J.H. 11/30/29. ♂ W. 4/6/31. ♀ Mrs. E.G. 6/27/32. ♀ M. 8/18/34. ♀ *D*. 5/3/38. Le. at 2 mos. ♂ R. 2/14/43. ♂ L. 9/30/49	3
		M	4	2	14	44, 78, 32	+	2	3	2		4, 0
70	6 Mc Negro	P	?	?	?	?	+	?	?		♂ *M*. 7/5/50. d. at 29 mos. Rt. at 2 yrs.	
		M	4	2	4	1	+	6	4			
71	7 Mc	P	4	2	14	23, 7	+	1			½ sis. by father. ♀ B. 7/29/37. ½ sis. by mother. ♀ J. 10/24/41. ♀ M. 5/12/43. ♀ P. 3/3/45. Full sibs. ♂ *D*. 12/18/51. Bilateral at 18 mos.	
		M	4	2	10	15	+	6	9			
72	8 Na	P	?	2	?	?	+	9	8	6	♂ *N*. 2/13/42. Le. at 2 yrs. d. 2 yrs. 7 mos. ♂ R. 7/4/43. ♂ J. 5/29/48. ♂ A. 10/6/50	
		M	?	2	1 (Others?)	3, 4, 1	+	5	7	1		
73	9 Ni	P	4	2	10	22, 14	+	3	6		♂ *R*. 1/31/56. Rt. at 3 mos. ♀ M. 12/7/57	
		M	4	2	5	14	+	4 (half)				
74	5 Og	P	4	2	14	28, 45 (8?), 7	+	3	9	3	♀ *Mrs. M.B.O.* 6/13/37. Unil. at 10 mos. has 1. ch. 5/5/56. ½ bro. by father. ♂ M.B. 6/8/49	
		M	?	2	10 (7?)*	10 (6?)*	+	2	7	1		
75	5 O'N	P	4	2	12	10	+	1			♀ M. 8/17/49. ♀ M. 10/11/51. ♀ M. 10/7/52. ♀ M. 3/20/54. ♂ *K*. 11/28/56. Bilateral at 11 mos.	
		M	4	2	8	29, 54, 3	+	7	19			

* Although not traced, said to have no eye cancer.

TABLE 4—*continued*

Fam. No.	Family Name		G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des, P. S. C.	Sibship	Sibs'C
76	3 Ot	P	4	2	8	23, 24	+	5	7		♂ *G.* 1/2/54. Le. at 9 mos. ♂ A. 5/7/55	
		M	4	2	8	14, 5	+	1	2			
77	8 Ov See Fig. 1.	P	?	?	?	?	+	?	?		♀ *M.* 8/6/48. Rt. at 13 mos. Le. at 19 mos. d. 26 mos. ♀ *P.* 2/11/51. Bilateral at 11 mos. ♂ W. 8/3/52	
		M	4	2	9	12 (?)*	+	1				
78	8 Ov	P	4	2	9	6, 9	+	4	6		♂ G. 7/27/52. ♂ *S.* 9/12/55. Le. at 24 mos.	
		M	4	2	5	10, 6	+	3	6			
79	5 Pe	P	4	2	18	51, 63, 1	+	1	2		♀ P. 10/22/40. ♂ *R.* 7/17/46. Bilateral at 7 mos. ♀ 3/21/57	
		M	2	2†	4	4, 2	+	1				
80	5 Pe	P	4	2	16 (9?)		+	5	8	7	♀ Mrs. B.H. 3/6/26. ♀ Mrs. D.R. 3/25/27. ♀ J. 7/26/28. ♀ Mrs. J. C. 12/21/31. ♀ *S.* 7/2/32. d. 17 mos. Bilateral. ♂ R. 4/21/33. ♂ R. 6/1/35	3, 6 8 1
		M	4	2	12	49, 94, 104, 5	+	5	15	17		
81	5 Pe Negro	P	?	?	?	?	+	?	?	?	♂ J. 8/1/34. ♀ Mrs. E.C. 2/23/36. ♂ B. 8/26/37. ♀♀ Mrs. G.S. and *Mrs. M.F.* 8/9/40. Le. at 1 yr. Twins said to be identical. No conf. ♂ V. 9/29/44. ♀ J. 3/7/48	1, 2 2 1, 1
		M	4 (2?)	2	4	29	+					
82	7 Pr	P	4	2	7	21, 19	+	1	3		♀ C. 1/31/50. ♀ *L.* 6/12/51. Bilateral at 18 mos. ♀ S. 4/20/53. ♀ C. 10/30/54	
		M	4	2	12	16, 7	+	5				
83	5 Pr	P	4	2	13	28 (2?), 32, 16	+	5~	4		♀ A. 11/23/45. d. at 3 days. ♂ M. 12/30/46. ♀ *K.* 6/13/49. Le. at 22 mos.	
		M	4	2	6	18, 39, 1	+	2	2			

84	6 Qu	P	4	2	6	46, 3	+	4	2		♀ Mrs. M.R. 8/9/27. ♀ *A*. 10/13/32. Rt. at 8 mos. ♀ J. 9/15/35. ♂ R. 7/9/44
		M	4	2	8	15, 25, 7	+				
85	6 Ra	P	?	2	?	?	+	1	2		½ sibs by mother. ♀ S. 9/5/43. ♀ T. 9/6/46. ♂ M. 10/22/48. ½ sibs by father. ♂ L. 5/10/46. Full sib. ♂ *L*. 11/8/51. d at 25 mos. Bilateral at 20 mos.
		M	4	2	6	6, 4	+	3	2		
86	5 Ra	P	4	2	1	2	+	10			♂ T. 1/48. ♂ D. 4/49. ♂ R. 5/8/50. ♂ D. 1/17/52. ♂ *K*. 8/4/53. Le. 1 mo. ½ sis. by mother. ♀ R. 6/25/56
		M	4	2	4	17, 24	+	2 (half)			
87	7 Ra	P	4	2	20	28, 51, 5	+	7	23		♂ F. 11/24/33. ♀ B. 10/25/36. ♂ C. 2/7/39. ♀ *M*. 6/2/42. d. 3 yrs. Bilateral at 2 yrs. ♂ W. 3/19/45. ♀ E. 10/12/47. d. at 3 yrs. no eye ca. ♂ J. 11/4/48. ♂ R. 3/4/49
		M	4	2	6	9	+	5			
88	6 Re	P	?	2	?	?	+	5	12		♀ *C*. 2/26/47. d 4½ yrs. Bilateral at 20 mos. ♂ F. 12/14/50. ♂ D. 2/20/53
		M	4	2	10	22, 32	+	4	1		
89	6 Rh	P	4	2	8	19, 2	+	8	9	1	♀ *B*. 5/28/38. d 27. mos. Rt. at 17 mos. ♀ S. 3/20/41. ♀ H. 3/13/43. ♂ P. 10/11/54. ♀ M. 7/15/56
		M	?	2	3 (others?)	?	+	6	13		

* Not traced but said not to be blind.
† One an adopted child, no family history.

TABLE 4—*continued*

Fam. No.	Family Name	G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des, P. S. C.	Sibship	Sibs'C
90	4 Ro P	?	2	12?	19?	+	7	22 (2‡)	7 (3‡)	♂ J. 6/19/28. ♂ J. 11/25/30. ♀ Mrs. G.W. 11/19/33. ♀ P. 10/31/38. ♀ V. 2/2/47. ♂ *D*. 11/11/50. Le. at 5 yrs.	
	M	4	2	8	24 (11?), 16	+	8	17 (2‡)	13 (3‡)		
91	5 Ro P	4	2	17	49, 76, 2	+	10	11		♀ M. 1/25/46. ♂ *H*. 3/2/47. Rt. at 3 yrs. 10 mos. ♀ R. 9/16/52	
	M	4	2	5	1	+	4	6			
92	7 Ro P	4	2	7	4	+	1	1		♂ *S*. 6/51. Le. at 39 mos. ♂ J. 6/54. ♀ ♀ J. and J. 1/9/57	
	M	4	2	6	18, 27	+	5	11			
93	4 Ro P	4	2	15	16	+	3	7		♀ C. 6/14/46. ♀ J. 6/25/47. ♂ *R*. 10/12/53. Rt. at 2 wks.	
	M	4 (2?)	2	2	2	+	1	3			
94	5 Ru P Negro	?	2	4 (others?)	2	+	1	?		♀ R. 3/24/43. ♀ M. 12/2/44. ♂ *R*. 7/14/46. Rt. at 8 mos. ♂ D. 12/14/47. ♀ V. 5/19/54.	
	M	4	2	6	3, 5, 13	+	15	28			
95	5 Sa P	?	2	2	7?	+	7	26		♂ *D*. 2/28/45. Le. at 2 yrs. ♂ R. 5/11/48. ♂ J. 4/28/51. ♂ R. 5/3/54. d. 2 days. ♀ M. 6/2/55	
	M	4	2	12	29, 26	+	3	11			
96	7 Sa P	?	2	3	2	+	4	3	3	♂ K. 2/8/41. ♂ R. 9/2/42. ♂ *R*. 1/13/44. Rt. at 46 mos. ♀ S. 9/30/48. d. at 9 yrs. no eye ca.	
	M	?	2	1	1	+	7	13			
97	7 Sc P	4	2	12	23, 30	+	4	5		♀ *R*. 1/13/48. Bilateral at 6 mos. ♀ B. 12/13/52. ♀ D. 8/26/55	
	M	4 (2?)	2	8 (7?)	4, 1	+	5 (half)	5			

‡ Double first cousins and children of these. Same as those on maternal side.

98	6 Sc	P	4	2	7		+	2 (half) 1 (full)	4		♀ *K*. 5/17/42. Rt at 3 mos. ♂ F. 9/17/56
		M	4	2	3	11, 10, 3	+	2	6		
99	5 Sc	P	4	2	11	18, 28, 2	+	3	4		♀ *L*. 17/18/47. Le. at 1 yr. ♂ N. 9/28/48
		M	4	2	6 (3?)	19, 12	+	2	3		
100	5 Sc	P	?	2	1 (others?)	6	+	8	16		♀ E. 5/23/40. ♀ S. 11/15/42. ♀ *W*. 6/2/50. d. 38 mos. Le. at 24 mos.
		M	4	2	10	26	+	5	7		
101	7 Sh	P	4	2	12	20, 16, 7	+	3	6		♀ *B*. 12/5/46. d. at 4 yrs. 3 mos. Eye removed at 3 yrs. ♂ D. '48. ♀ J. 8/50. ♂ G. '54. by 2nd husband ♂ M. 9/29/55. by 3rd husband ♂ R. 11/4/56
		M	4	2	13	23, 9, 1, others?	+	7	10		
102	8 Sh	P	4	2	4	1	+	2	3		♀ *R*. 9/30/54. Rt. at 30 mos. ♂ G. 11/16/55. ♂ T. 2/18/58
		M	4	2	12	18, 15	+	1			
103	5 Si	P	?	2	1 (others?)	4 (others?)	+	4	7	6	♂ *N*. 4/25/38. Rt. at 2 yrs. ♂ J. 5/5/42. ♂ R. 3/37/46. ♂♀ J. and J. 6/27/53
		M	4 (2?)	2	9	23, 29, 1	+	6	5		
104	8 Sm	P	4	2	12 (9?)	6	+	2	1		♀ *M*. 6/30/43. d. at 3 yrs. Rt. at 15 mos. ♀ T. 6/11/46. ♂ J. 4/19/48. ♀ P. 4/28/49. ♀ S. 12/24/52. ♂ M. 2/1/53
		M	4	2	9 (7?)	5	+	6	5		

TABLE 4—*continued*

Fam. No.	Family Name		G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des. P. S. C.	Sibship	Sibs'C
105	5 Sm	P	4	2	14	68, 113,* 92	+	7	21	5	♀ S. 1/13/50. ♀ *S*. 10/23/51. Rt. at 51 mos. ♂ J. 5/21/54. ♀ S. 9/30/55	
		M	4	2	14	61, 48†	+	12	21			
106	4 So	P	4	2	9	20, 6	+	9	5		♀ C. 9/19/47. ♀ *B*. 11/16/48. Rt. at 2 yrs. Le. at 26 mos. ♂ J. 2/22/49. ♀ S. 1/21/50. ♀ N. 4/24/51	
		M	4	2	9	34?, 47?	+	2				
107	6 So	P	4	2	14	38, 57, 7, 1	+	6	14	7	♀ Mrs. N.B. '30. ♀ Mrs. C.S. '32 Mrs. A.S. '35. ♂ C. '36. ♂ J. '39. ♀ *J*. 3/14/42. Onset 2 yrs. side?	1, 2
		M	4	2	?	?	+	1	7			2, 1
108	9 St.	P	4	2	4	1, 3	+	1			♂ *F*. 3/3/57. Bilateral at 2 wks.	
		M	4	2	4	14, 26, 4	+					
109	5 St	P	4	2	13	6, 6	+	7	10		♀ *L*. 1/28/48. Le. at 32 mos.	
		M	4	2	9	6, 3, 2	+	1	1			
110	7 Te	P	4	2	17	15, 4, others?	+	4	12		♀ J. 5/18/47. ♂ *T*. 9/15/50. Bilateral at 11 mos. ♂ W. 2/4/53	
		M	4	2	2	2, 6	+	1				
111	6 Th	P	4	2	19	28, 49, 6	+	5	17		♀ *P*. 4/18/46. d at 31 mos. Bilateral at 8 mos. ♂ D. 4/10/47. ♀ V. 7/18/48. ♀ P. 7/11/50	
		M	4	2	5	7	+	4	5			
112	8 Th Negro	P	?	2	3	?	+	8	18		♂ J. 5/5/48. ♂ *D*. 4/26/49. Bilateral at 5 mos.	
		M	4 (2?)	2	5	1, 1	+	3				

113	5 To	P	4	2	11	28, 36	+	5	1		½ sib by father. ♀ P. 10/1/39. ½ sib by mother. ♀ E. 2/2/41. Full sibs. ♂ *J*. 6/20/44. Rt. at 2 yrs. Le. involved. ♀ E. 6/22/46. ♀ J. 3/16/50. ♂ P. 5/1/52	
		M	4	2	6	2	+	1	1			
114	7 Tu	P	4	2	10	28, 45	+	3	5		♀ *C*. 8/25/46. d. 39 mos. Unil. at 2 yrs. ♀ 8/25/51. ♂ L. 11/29/56. ♂ T. 11/8/57	
		M	4	2	9 (7?)	12, 13	+	3	3			
115	3 Ul See Fig. 2.	P	4	2	11	50, 26	+	8	36	6	♀ D. 8/4/52. ♀ B. 3/8/54. ♂ *R*. 12/21/55. Bilateral at 3 wks. ♀ *S*. 4/11/57. Le. at 3 mos. ♀ *N*. 2/59. Rt. at 3 mos.	
		M	4	2	11	41, 27 others?	+	11	22			
116	11 Van	P	4	2	15 (2?)	47, 54	+	2	2		♀ E. 12/17/47. ♂ *R*. 2/21/51. Rt. at 25 mos.	
	Paternal side not shown in figure. See Fig. 8.											
		M	4	2	8	39, 64	+	3	7			
117	10 Van	P	4	2	12	15, 31, 3	+	4	6		♂ *H*. 5/21/32. d. at 2 yrs. Bilateral, onset? ♀ C. 6/10/36. ♂ K. 1/25/37	
		M	4	2	2	11, 15 (8?)	+	3	6	1		
118	7 Va	P	?	2	?	?	+	1	2		♀ Mrs. J.R. 11/10/28. ♂ L. 8/23/30. ♀ D. 10/24/32. ♀ R. 4/37. ♀ *V*. 1/21/51. Rt. at 4 yrs.	2
		M	?	2	?	?	+	9	11	7		
119	5 Vi	P	?	2	?	?	+	1	4	1	♀ J. 2/8/40. ♀ *J*. 8/13/45. Le. at 14 mos.	
		M	?	2	5	4, 3, 4	+	8	12			
120	4 Wa Negro	P	?	2	?	?	+				♀ '50. ♀ '51. ♀ *E*. '52. Unil. at 2 yrs. ♀ '53	
		M	4	2	?	?	+	9 half				

* An additional unknown number not traced.

TABLE 4—*Continued*

Fam. No.	Family No.	G. G. P.	Gr. P.	Gr. P. S.	Des. Gr. P. S.	Par.	P. S.	P. S. C.	Des P. S. C.	Sibship	Sibs'C
121	6 Wa P	4	2	9	28, 38	+	3	6		♀ *J.* 9/25/37. d. at 4 yrs. Rt. at 3 yrs. ♀ C. 3/7/41. ♀ J. 8/4/42. ♂ R. 12/30/43. ♀ M. 4/17/46. ♀ N. 5/3/47. ♂ J. 2/14/50. ♀ C. 1/25/52	
	M	4	2	10	27, 42, 50, 6	+	4	8	2		
122	5 Wa P	4	2	11	19, 17, 2	+				♂ T. 1/7/38. ♀ K. 4/18/43. ♀ *S.* 11/27/45. Le. at 8 mos.	
	M	4	2	6	16, 1	+					
123	7 We P	4	2	17	9	+	1	2		♂ J. 1/8/42. ♂ R. 8/11/43. ♀ *L.* 2/2/46. Unil. at 21 mos.	
	M	4	2	4	4, 4	+					
124	5 Wh P	4	2	11	7, 17	+	1			♂ M. 7/7/52. ♂ *R.* 8/20/53. Bilateral at 6 wks.	
	M	4 (2?)	2	5	3, 8	+	9	21			
125	6 Wi P	4	2	10 (4?)	16, 36	+	3 full 4 half	41	11	♂ L. 6/23/42. ♂ *S.* 11/9/44. d. at 41 mos. Bilateral. ♂ D. 11/22/46. ♀ L. 3/3/48. ♂ D. 3/17/51. ♀ P. 6/3/53. ♂ R. 12/8/55	
	M	4	2	9	48, 25 (9?)	+	8	23	4		
126	6 Wi P	4	2	12	44, 90, 17	+	5	16	2	♀ J. 3/11/49. ♂ *E.* 1/6/51. Le. at 40 mos. ♂ S. 6/6/55	
	M	4	2	10	32, 53	+					
127	7 Wi P	4	2	22	50, 115, 36	+	5	9		½ sibs by father. ♀ ♀ S. and N. 4/3/44. Full sibs. ♀ *D.* 7/20/50. Eye removed at 5 mos. ♂ D. 1/1/55	
	M	4	2	16	24, 39 (3?), 13 (3?)	+	6	8			
128	7 Wi P	4	2	9	21, 45	+	2	5		♀ D. 8/13/55. ♀ *D.* 2/16/57. Rt at 6 wks.	
	M	4	2	6	39, 30	+	1				
129	7 Wi P	4	2	6	12, 19	+	4	13		♂ K. 2/28/51. ♂ D. 2/24/53. ♂ *W.* 5/25/54. Le. at 3 yrs.	
	M	4	2			+	5	5			

130	5 Wo Negro	P	?	2	3 (others?)	1 (others?)	+	4			♂ D. 9/1/44. ♀ *L.* 6/25/52. Rt. at 20 mos. Le. at 4 yrs. Rhabdomyosarcoma knee at 5 yrs. ♂ F. 3/38/55	
		M	4	2	4		+	16	20	5		
131	4 Wo	P	?	2	10	40?	+	5	11		♀ Mrs. C.F. 9/21/30. ♂ *A.* 11/29/35. Bilateral at 14 mos.	3
		M	?	2	5?	7, 14	+	4	5	5		
132	5 Wo	P	4 (2?)	2	2	8, 15, 12	+	2	1		♂ M. 5/31/54. ♀ *N.* 3/23/56. Rt. at 13 mos. Le. at 16 mos.	
		M	4	2	9	16 (9?)	+	8	21			
133	7 Wo	P	4	2	13	15, 21, 1	+	5	11	4	♀ Mrs. T.P. 8/28/36. ♀ J. '37. d. 2 mos. ♂ T. 9/30/39. ♂ D. 5/9/41. ♂ D. 3/28/43. ♂ M. 1/10/49. ♀ *R.* 8/15/53. Rt. at 22 mos.	1
		M	4	2	18	56*, 41, 16	+	5	12			
134	6 Wr	P	4	2	6 (3?)	6, 11 (5?)	+				♂ *D.* 2/11/47. Le. ♂ C. 12/25/48. ♂ G. 7/12/51. ♂ M. 2/12/53	
		M	4	2	10	35, 67, 9	+	3	8			
135	5 Yo	P	4	2	11	30, 57	+	3	4		♀ *C.* 8/2/52. Rt. at 25 mos.	
		M	4	2	15	52, 50	+	2	3			

* Nothing known of descendents of 31.

Explanation of Abbreviations in Table 4

Family Name. The numerals before the letters indicate the number of letters in the surname. The letters are the first two of the name. Thus 5 Bl would stand for Black, Blank, etc.

G. G. P. = great grandparents. *Gr. P.* = grand parents. *Gr. P. S.* = sibs of the grandparents. *Des. Gr. P. S.* = descendants of sibs of the grandparents, with the numbers indicating the number of relatives traced in subsequent generations. *Par.* + = parent contacted. *P. S.* = sibs of parent. *P. S. C.* = children of parent's sibs or first cousins. *Des. P. S. C.* = descendants of first cousins. *P* = paternal relatives. *M.* = maternal relatives. *Sibs'C.* = Sibs' children.

Sibship. ♂ H. a brother whose name begins with H. It is followed by his birthdate. ♂ *R.* a proband, name beginning with R.

12 (8?) = 12 persons, 8 of them not traced. Blank spaces mean that there are no relatives in that category. Families with no ? and no statement that there is no information are complete for all relatives. Number of relatives in the table will most often not be the same as in the figures, which have been drawn as skeleton outlines to make the relationships and number of relatives carrying the gene non-penetrant clear.

further interesting facts about this family. The mother of I-2 in Fig. 4 was a sister of I-4 in Fig. 5. This meant that all four lines of affected relatives were descended from three families, the Lu, the Bl and the St lines, the mother of I-2 in Fig. 4 being an St. It was also shown that all four affected lines were likewise descended from another family whose surname was Ma. Now the relationship between IV-1 and 2 in Figure 4 became clear. This new relationship is shown in Figure 5, where all known consanguineous marriages in the Ma line are indicated by double mating lines.

Only a small part of this family has been traced, although over 4000 relatives have been written to, or interviewed personally, or their parents have been interviewed. Figure 5 shows that IV-1 was descended from one of two Ma brothers. Her husband (IV-2) descended from the other brother, (I-6) on the paternal side, and from a woman who was said to be their sister on his maternal side. Since this relationship is not certain, no vertical line is drawn from the sibship line of I-1 and 2. Her maiden name, however, was the same as their surname. I-6 in Fig. 5 had two wives, and 33 legitimate children and at least 4 illegitimate children who were known to his grandchildren.

The names and some of the descendants of eight of the legitimate children were secured, but they are not shown in Figure 5 since no retinoblastoma was found among them. Three of the legitimate sons and some of their descendants are shown, although again many descendants have been omitted because of no evidence of the disease. This family would make one suspect that there are two genetically different retinoblastomas, the gene being dominant in most families, but recessive in this one. The objection to such an interpretation is that the proband VI-1 in Fig. 5 is not the product of a consanguineous mating. If this family exhibits a recessively inherited condition, V-9 would have to be heterozygous. Most workers agree that retinoblastoma is not a recessive trait, therefore, the recessive gene, if it exists, must be extremely rare. It is highly improbable that a man from a family in which this very rare gene should have already exhibited itself should marry a woman carrying the same gene. It is not impossible, but is less probable than the likelihood that the gene in this family exhibits varying degrees of penetrance, being highly penetrant in two of the four affected sibships, but poorly penetrant in most of the family and in one sibship in which only one of eight children showed the disease.

Families 55 and 62 are shown in Figure 6. Both V-3 and VI-7 were probands in separate families. Neither set of parents knew of the other affected proband. The death certificates of III-3 and III-2 showed that they were brother and sister. This family illustrates the value of obtaining death certificates of all relatives even though they were known not to have the disease in question.

Family 14 is shown in Figure 7. It is one in which a seemingly sporadic case was found to have an affected relative. VI-5 was the original proband. The mother had had one illegitimate child, (the dotted line in the figure should have extended from the dotted mating line) then 15 others by her first legal husband, and then two by her second husband. The second child of the third union developed retinoblastoma. In investigating the father's family (the mother's was

also searched for), there was no evidence of any other child with the same condition. While in Kentucky, trying to find the family of IV-5 in Figure 4, the field worker encountered the grandmother of VII-4 in Figure 7. She had heard that this woman had a grandchild who had died with the disease. Inquiry into the family history showed that there was no relationship to the Lu Family. The family history of the grandchild was collected as a new project after the death certificate was located, stating that the child had died of bilateral retinoblastoma. As the inquiry proceeded, it became evident that the grandmother of the woman being interviewed was the sister of the grandfather of the third husband in the Ohio Family 14. No other cases in either Kentucky or Ohio were found in this family. At the time that the family of the first proband was being traced, the author felt certain that if a collateral line was discovered to have the disease, it would be on the father's side, since the mother had already had 17 opportunities of passing on a gene which she might have been carrying nonpenetrant, and had failed to do so. The finding of the second case here was chance, since it was further removed from the original proband than the investigations usually went.

Family 116 is shown in Figure 8. Here two second cousins have the disease, one bilaterally, the other in the right eye. The mother (IV-2) of the proband stated that two of her cousins each had a child with retinoblastoma. Correspondence with the other branches of the family secured the pathological diagnosis of retinoblastoma in V-4, but the family of V-9 refused to answer. Finally the

DESCRIPTION OF FIGURES

In all figures, squares are males; circles, females. A completely black symbol shows bilateral involvement, a half black symbol a unilaterally affected person, either right or left eye affected. Symbols with figures inside represent a total of that many normal persons. Pedigrees seldom include the full number of relatives investigated, since this is shown in Table 4. When the side of the family through which the gene is transmitted is known, the other side of the family may not be shown at all, although given in full in Table 4. The pedigree is reduced to its simplest outline to make relationships obvious.

The generation numbers in the table do not necessarily correspond to the generation numbers in the pedigrees, which were drawn in most cases to show the latest generation in which the mutation could have occurred in order to be reproduced in the affected persons. This was for ease in counting the number of persons through whom the gene passed nonpenetrant. The first generation in the pedigrees may be one on which no information was secured.

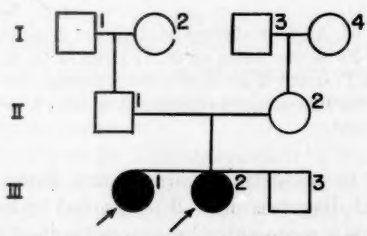

FIGURE 1. Fam. 77. Two sibs affected. Paternal side of family unknown father being an adopted child. Generation I in figure the latest in which mutation could have occurred.

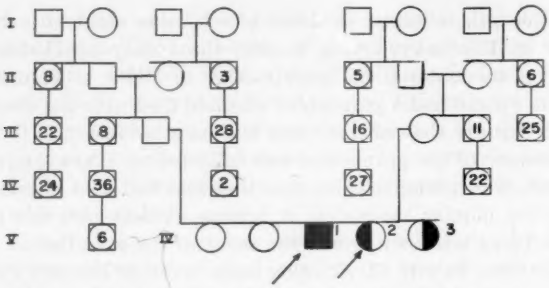

FIGURE 2. Fam. 115. Two affected sibs at time of interview. A fifth child, born in Feb. 1959, showed retinoblastoma in right eye in May 1959.

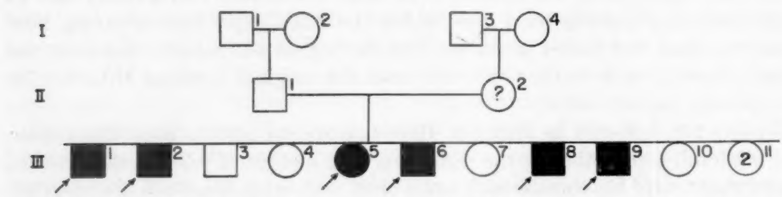

FIGURE 3. Fam. 12. Six in a sibship of ten affected with bilateral disease. Two miscarriages. Mother has microphthalmic eye, no vision, refuses examination. Generation I latest generation in which mutation could have occurred.

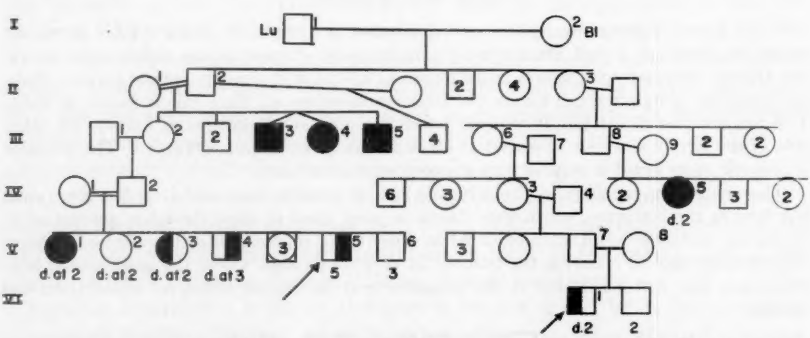

FIGURE 4. Fam. 60 and 63. All four affected sibships are descended from the Lu, and Bl, the Ma and the St lines, I-2's mother being an St. II-1 and -2 are first cousins once removed, I-1 being first cousin to I-2. IV-1 and -2 are third cousins through the Ma line, shown in Figure 5. Over 4000 relatives of the four affected sibships have been traced, but no other affected persons have been discovered.

field worker was sent to southern Tennessee, and found the family, and obtained the pathological diagnosis on V-9. It proved to be glioma of the pons, not retinoblastoma. This is represented by a cross hatched square in the pedigree.

Family 52 is represented in Figure 9. The mother's side of the family is re-

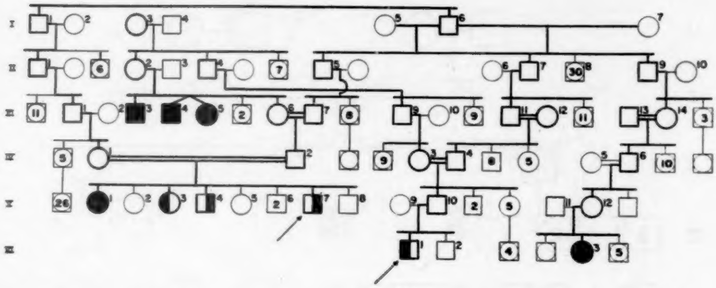

FIGURE 5. Fam. 60 and 63. The relationships are now traced through the Ma line, descent in this line being shown in heavy black lines. I-3 was an Ma, probably a sister of I-1 and I-6. Related parents are joined by a double mating line. Fully a third of the matings in this family are between persons related through one of the four lines from whom all affected children are descended. It has been impossible to show that the four lines all stem from a common ancestor.

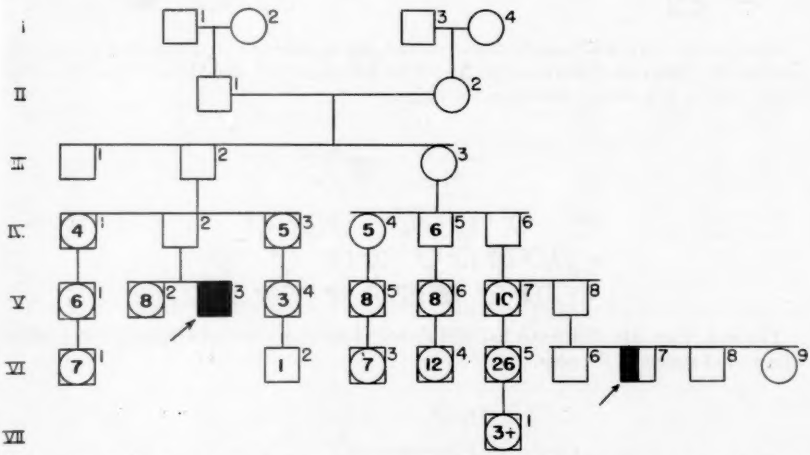

FIGURE 6. Fam. 55 and 62. The probands were in two families not known to be related at the time of the interview.

corded in Table 4. The proband was the first of two children, and no other case was known in the family. A second case was uncovered in a second cousin of the proband. This child had been operated upon for unilateral retinoblastoma some months before. Again what appeared to be a sporadic case proved to be one that was probably inherited.

Family 67 is shown in Figure 10. Two sisters each have produced a child with retinoblastoma. Both children were secured as probands from the hospital in which they were treated. The complete family history is given in Table 4. Neither husband was related to his wife, and they were not related to each other.

III-5 reported that her sister's boy who had been a unilateral case at the

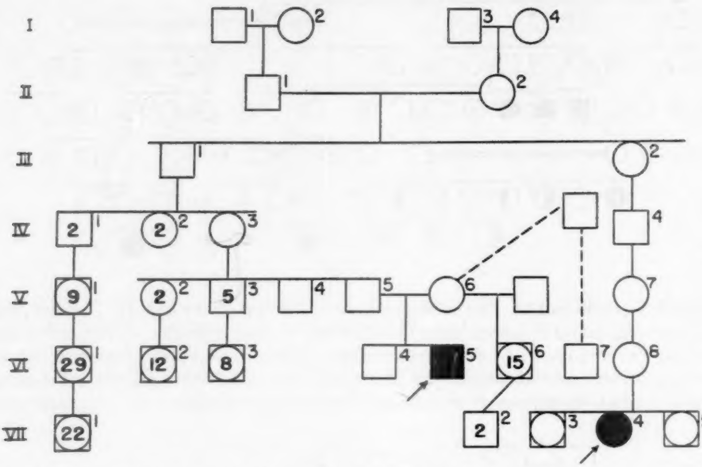

FIGURE 7. Fam. 14. VI-5 was the Ohio proband. VII-4, unknown to the parents of VI-5, was accidentally discovered in Kentucky. The dotted vertical line should have come from the dotted mating line, rather than from the father.

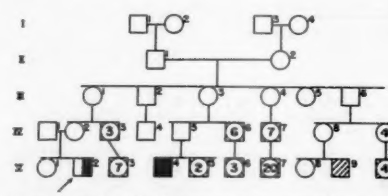

FIGURE 8. Fam. 116. The cross hatched square represents a second cousin of the proband who died of glioma of the pons.

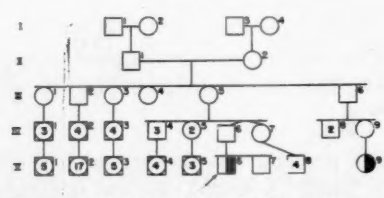

FIGURE 9. Family 52. The parents of the affected children were unaware of the disease in their cousin's child.

time of interview had developed the disease in the other eye, and was given only a few months to live.

Family 40 is shown in Figure 11. The father's maternal uncle was affected. A death certificate was obtained stating that he had died at the age of two years following an operation for cancer of the eye. Again a seemingly sporadic case proved to be otherwise, if one accepts the most probable explanation of this death certificate.

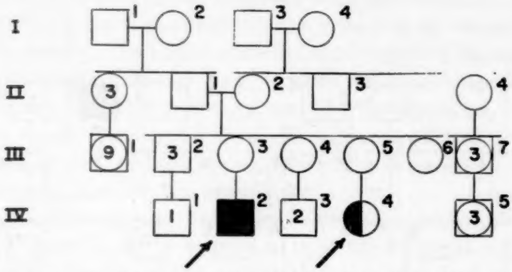

FIGURE 10. The pedigrees of the two fathers of the affected cousins are not shown, nor is the family history of the father of IV-2 recorded in Table 4, since the gene was obviously being carried by the two mothers who were sisters. Family 67.

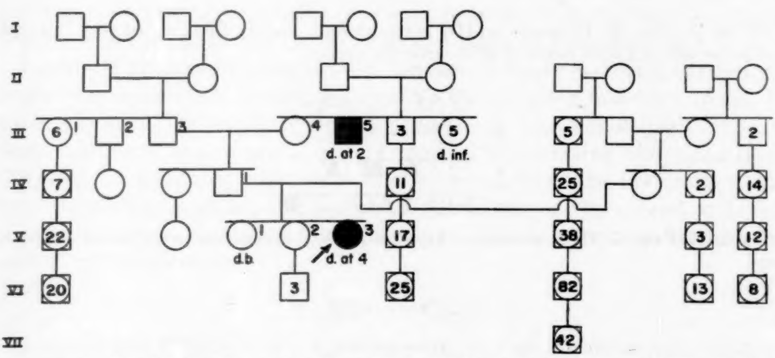

FIGURE 11. Family 40. The proband was first considered to be a sporadic case until her father's maternal uncle was found to have died of cancer of the eye.

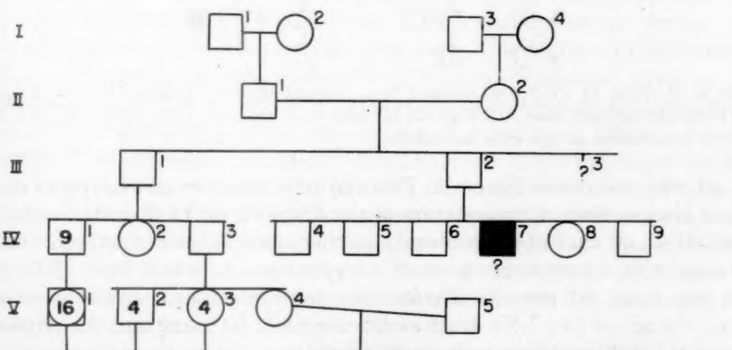

FIGURE 12. Family 65. Diagnosis on IV-7 not confirmed. Family reported eye cancer with death in early childhood in this boy.

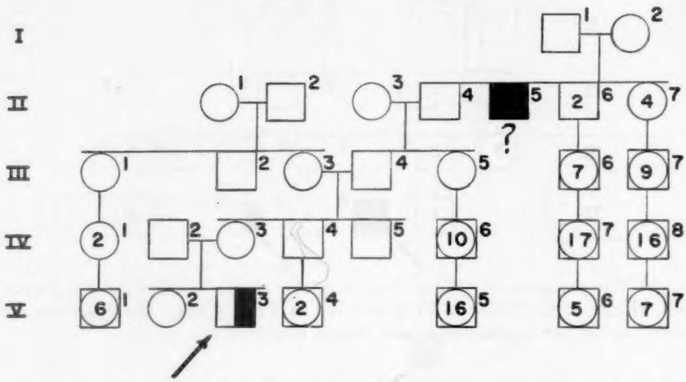

FIGURE 13. Fam. 23. Diagnosis on II-5 not confirmed. Family Bible stated that this child died at the age of 2 after removal of the eyes for cancer.

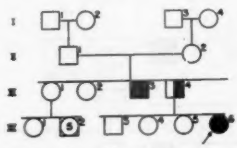

FIGURE 14. Fam. 47. III-4, although a hereditary case, has remained a unilateral one for 43 years.

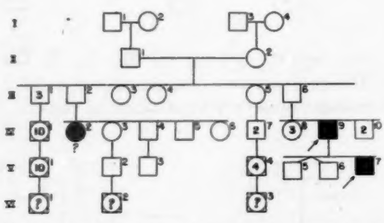

FIGURE 15. Fam. 41. IV-9 was obtained as a proband after his daughter had been found. IV-2 is an unconfirmed case, although all her sibs state that she died at the age of 2 after bilateral enucleation of the eyes for cancer.

Family 65 is shown in Figure 12. This may or may not be an example of an inherited disease, since a confirmation of the diagnosis on IV-7 could not be obtained. IV-2, an aunt of the proband's mother, stated that her father's brother had a son with a very large tumor of the eyes when he was a small child, that "His eyes hung out over his cheeks, they were so swollen, and that he died around the age of two." No death certificate could be found and the physician treating the child was long since dead.

Family 23 (Figure 13) was regarded as representing a sporadic case when a second call for other information upon a paternal aunt of the proband's mother elicited the fact that some of her living uncles had a family Bible with records

in it. This led to further investigation into lines not ordinarily studied because of lack of knowledge of these more remote relatives on the part of the living members of the family. The record stated that a brother of the mother's paternal grandfather had died at the age of four after removal of the eyes for cancer. There is no confirmation that this was a case of retinoblastoma, hence it has been queried in the figure. The age at which the disease occurred, the fact that the eyes had to be removed, and the appearance of the disease in a relative make it quite probable that it was retinoblastoma.

The next two families are the only ones in which parents of the probands were also affected. Figure 14 shows a small part of the paternal side of Family 47. III-3 was an older brother of the proband's father who had died at the age of 4 with bilateral disease existing since the age of two. The father's right eye only was affected; he had survived for 43 years without the other eye becoming diseased. At the time of his marriage he had consulted a physician who assured him that this was not a hereditary case, despite the fact that he had an older brother who died of it. His fourth child had bilateral retinoblastoma.

Family 41 (Figure 15), was another instance of father and child affected. The father was ascertained independently after the daughter had been found. His paternal cousin died at the age of two, but it has been impossible to obtain a death certificate or a record of her operation. The remaining sibs in the family (IV-3–6) all state that their sister died with cancer of the eyes after having been operated upon by a Columbus surgeon, who has long been dead, and whose office records were destroyed many years ago. This may or may not be a true case of retinoblastoma.

PENETRANCE

The problem of penetrance is linked with that of mutation rate, since the lower the penetrance the lower the mutation rate may be. If the gene is almost fully penetrant each sporadic case that is bilateral and some of the unilateral cases will be apt to be dependent upon a germinal mutation. If the gene has a low penetrance many cases may appear to be sporadic that are not so, hence they will increase the estimated mutation rate. The degree of penetrance assigned to the gene for retinoblastoma has varied from 78 per cent (Franceschetti) to 98.5 (Neel and Falls). Neel gives a formula for arriving at a rough estimate for failure of penetrance. First one deducts all families in which a parent is affected. Then one takes the ratio of index cases with affected sibs to all index cases, both sporadic and with sibs affected. In his data there were 58 families with 64 sibships. In five of these the parent was affected. In four of the remaining 59 sibships with normal parents there were affected sibs. The final result was 1/67 or .015 for failure of penetrance, making the degree of penetrance 98.5. He thinks that this estimate may be a little high and that the degree of penetrance may be nearer to 95 per cent.

If this method is applied literally to the present study, calling those cases sporadic in which neither a parent nor a sib is affected, although other collateral branches are affected, one finds that there would be 130 cases listed as

sporadic, and eleven index cases found in four families where other sibs are affected. The figure for non-penetrance would be 11/141, or 7.8 per cent. If, on the other hand, one contrasts the families that appear to be truly sporadic with those in whom some relative not a parent is affected, the present series furnishes 22 hereditary cases against a total of 141, giving a percentage of non-penetrance of 15.6.

Inspection of the families whose pedigrees are shown in Figures 1 through 15 would lead one to question penetrance as high as 95 per cent. An ideal method, if it were possible, would be to determine all those who carried the gene and all those who carried the gene and showed the disease, and to estimate from these figures the degree of penetrance. Unfortunately, one usually does not know how many persons have carried the gene before it becomes evident as the disease in the sporadic cases. One can determine, when two or more collateral branches of a family are affected, what must be the minimal number of persons through whom the gene must have been transmitted if the two affected persons are considered as bearers of the same mutant gene. It is possible that in a rare instance two persons supposed to have derivatives of the same mutant gene from some common ancestor, are, in fact, instances of independent mutations. That this should occur as frequently as it appears to have done in this group of families seems to be most improbable.

In Figures 1 and 2 the gene must have passed non-penetrant through at least one person to have appeared in two offspring. Similarly, it must have been non-penetrant in at least one person to have appeared in six offspring in Figure 3. In Figure 4 it must have mutated at the latest in a parent of I-1 or I-2 to have been present in segregating form in either I-1 or I-2. If then one counts the number of persons through whom it passed in order to show up in the four collateral lines, and if one assumes that on the average it should appear in half the offspring of a parent carrying the gene, we have 30 carriers of the gene but only nine showed it, making the penetrance 30 per cent. If it is assumed that the sibs of the carriers should not be included, it went non-penetrant through nine persons to show up in nine, a penetrance of 50 per cent.

Studying the family from Figure 5 as if the gene had descended not through the Lu, the Bl or the St lines, but through the Ma line one finds that the number of persons through whom the gene went non-penetrant to be evident in nine persons varies from 37 to over 40, if the half of the sibs of carriers are regarded as carrying the gene also. It has been pointed out that two of the four sets of parents of affected offspring are related, but although as shown in Figure 4, II-1 and 2 are related as far as the St line is concerned, they do not appear to be related if the gene was transmitted through the Ma line. The affected sibship of four (V-1–8) comes from the Ma line through the mother, and from the Ma line through the father's father and mother. The degree of penetrance in this family, if the gene is to be regarded as a dominant, and if the four sibships are not to be thought of as caused by independent mutations, ranges from below 25 to about 30 per cent.

If all the families shown in the figures are counted, omitting Figure 5 which deals with the same family as Figure 4, and if the number of affected and

normal sibs are counted in each sibship in which an affected child occurs, one finds 44 affected children plus two who had to be carriers and 88 sibs who were normal. This gives a penetrance of 34.3 per cent, a figure much lower than that reported by other workers. Using the Yates correction in estimating $\chi^2_{(1)}$ gives a value of 12.54 ($P < .001$) if it is calculated on the basis of full penetrance, and a value of 5.74 ($P < .02$) if only 90 per cent penetrance is expected.

If the estimate of penetrance is made from those normal sibs who must have been carriers in order that the mutant gene from a common ancestor appear in two collateral lines, the degree of penetrance is much lower. In four families, the gene may have been transmitted from either the paternal or maternal line, thus making a different number of sibs to be counted as normal. Using the maximal number of sibs it was found that there were 37 who had to be carriers and 163 who were normal, making a penetrance of 18.5 per cent. If the minimal number of sibs is used there were 37 carriers and 148 normal sibs, giving a penetrance figure of 20.0 per cent.

It would appear that in the families in which some estimate of penetrance is possible the average figure is very much lower than that advanced by other workers. The degree of penetrance of any partially penetrant gene in man must have rather ill-defined limits, since the genetic background in which the gene operates is different in all families and in individuals within a family except in the case of identical multiple births. In this series, the gene was probably fully penetrant (if a dominant) in Family 12 in the sibship, and also in sibships of three and four affected in Family 60, while being markedly reduced in penetrance in the rest of Family 60. The average degree of penetrance deduced from one author's series of cases may well differ from the average in another author's series, not only because of the extent to which the family history is investigated, but because the degree of penetrance is a variable with a wide range.

If this investigation had stopped where many of the studies of this type do terminate, namely with the grandparents, parents, aunts, uncles, cousins and sibs of the affected child, the only families listed as showing hereditary retinoblastoma would have been those in Figures 1, 2, 3, part of Figures 4 and 5, with the two single sibs each being counted as sporadic cases, Figures 8, 10, 14 and 15. Twelve cases listed in the figures would have been classed as sporadic instances of the disease.

It is impossible to state what factors make the gene so highly penetrant in some families, and so low in penetrance in others. Despite the low degree of penetrance, and the fact that a large percentage of families continue to produce more children without their developing the disease, the tragedy to the child whose case may be bilateral, should the parents persist in having more children after one has become affected, would appear to justify counselling the cessation of child-bearing on the part of the parents, and to advise those affected cases, who live to maturity, to refrain from producing offspring.

MUTATION RATE

Several estimates of the mutation rate for retinoblastoma have been made. Philip and Sorsby (cited by Falls and Neel 1951) estimated that it was 1.4×10^{-5}

for England; Neel and Falls (1951) that it was about 2.3×10^{-5} for Michigan, with 95 per cent confidence intervals of 1.7 to 3.1×10^{-5}. Vogel's (1954) estimate was much less, being $.4 \times 10^{-5}$. If one considers that all the sporadic cases are examples of germinal mutation, the estimated mutation rate will be high. On the other hand, if one believes that many of the seemingly sporadic cases are such because the degree of penetrance is low, and that the germinal mutation has probably occurred some generations earlier rather than in the germ cells which made up the population under study, the estimated rate will be much lower. It is difficult, if not impossible, to decide which sporadic cases, especially the unilateral ones, may be germinal mutations, and which may be somatic mutations or phenocopies. Because the bilateral cases are much more frequent among the hereditary group, it is usually assumed that the bilateral sporadic cases are germinal mutations. It is possible to set two arbitrary limits to the mutation rate in a given population; the highest being obtained by counting all sporadic cases as germinal mutations, the lowest by counting only the bilateral sporadic cases.

It is quite evident that some unilateral cases belong in the hereditary group whether as germinal mutations occurring in the germ cell of an unaffected parent, or as the result of previous mutations some generations earlier, because persons who have remained affected in only one eye have reached maturity, married and produced bilaterally or unilaterally affected offspring. It is reasonable, therefore, to conclude that some of the unilateral sporadic cases are in truth germinal mutations, not somatic mutations, nor phenocopies. When one considers that a somatic mutation, to become evident as the disease, must arise in the cells of the organ which can show the disease, it is amazing that somatic mutations for retinoblastoma should arise so frequently in the cells of the retina that they are more numerous than the hereditary cases, in which the mutation is limited to a germ cell.

In estimating the possible mutation rate in Ohio, all hereditary cases had to be discarded, since the mutation did not involve the germ cells which made up the present population. All sporadic cases which were born outside the state of Ohio had to be excluded. The mutation rates might be different for the white and Negro races, so that the number of births and the number of retinoblastoma cases in the two groups had to be treated separately. Although the plan at the beginning of the study embraced only the years from 1940 through 1953, the number of cases found after that, and the delay in preparing the study for publication permitted the years 1954, 55 and 56 to be included. This gave two and a half years after the end of 1956 for children born in that year to develop the disease.

The children dying under one year of age are not likely to have had the disease and to have died of it, so that the number of deaths under one year was deducted from the total number of births for the year, to estimate the number of children in whom the disease might appear.

During the 17 years under study there were 2,720,929 white children born in Ohio who lived as long as one year. There were 92 white children born in Ohio who appeared to be sporadic cases, and whose names had been encountered. This

of course, was a minimal number, since not all such cases might have been found, although probably a very large percentage was obtained. This means that one in every 59,150 genes was mutating, or a rate of 1.60×10^{-5}. Similarly, there were 213,318 Negro children born during those years, who lived to be more than one year old. Among this group, 11 children with sporadic retinoblastoma were encountered. One in every 38,785 genes was mutating, a rate of 2.58×10^{-5}. Combining the two groups, the total mutation rate was 1.76×10^{-5}.

The mutation rate was higher among the Negro race than among the white. One explanation may be that the mutation rate is higher; another might be that physicians might not hesitate to send in names of clinicals cases, among which all the Negro children in this group would fall, while they might not be so likely to send in the names of private patients who might resent their names being given for a research project. Falls and Neel (1951) found the mutation rate somewhat less in the Negro than in the white.

If only the bliateral cases are considered, there were 33 of the 37 sporadic bilateral cases that were white, and 4 that were Negro. The mutation rate based on these figures would be 6.1×10^{-6} for white, and 9.4×10^{-6} for Negro. The combined rate is $.63 \times 10^{-5}$. This is doubtless too low. The real value probably lies somewhere between the first and second estimates given.

Fifty-eight point eight per cent of the hereditary cases were bilateral. If this figure is accepted as typical of this series and if one assumes that no non-hereditary cases are bilateral, one would expect that, in a group of 117 sporadic cases, the proportion of bilateral cases could vary between 58.8 per cent $\pm$ 2×4.55 per cent, the latter figure being standard error of the proportion. This gives upper and lower limits of 67.9 and 49.7 per cent of bilateral cases which could reasonably be expected among genetically determined sporadic cases. Thirty-seven bilateral cases were found among the sporadic instances of the disease. Equating 37 first to 67.9 per cent; second, to 49.7 per cent, gives a range of 55 to 74 of the 117 cases that might reasonably be expected to be hereditary, hence caused by germinal mutations. These figures give mutation rates of $.94 \times 10^{-5}$ and 1.26×10^{-5} respectively. The range of mutation rates runs from 1.76×10^{-5} when all sporadic cases are considered, to only $.63 \times 10^{-5}$ when only bilateral cases are considered, with 1.26 and .94 (both $\times 10^{-5}$) as intermediate estimates. The highest estimate is within the lowest range of Neel and Fall's figures for Michigan, (1.7×10^{-5}), and the lowest is not remote from Vogel's estimate ($.4 \times 10^{-5}$). Vogel's estimate is based upon an almost exclusively white population, while the combined rate here is for white and Negro, the latter having an appreciably higher rate. If one considers only the percentage of those sporadic cases which live who produce offspring showing the disease, the estimated mutation rate would be much lower. In this series there have been four affected persons who lived to reproduce. One in Family 47 was not a sporadic case since his brother also had the disease. A second in Family 41 thought himself to be a sporadic case since it was the investigation carried on by this study that uncovered the unconfirmed case in a cousin. Accepting him as a sporadic case, as he might have

been accepted in many studies, he produced an affected child. Two other cases, both unilateral, see Families 4 and 74 have married and each has had one child, neither of whom was affected at the time of the interview. The child in Family 4 was 13 months old two years ago, and the child in Family 74 was normal at 2½ years. The net result is that from among the 134 families interviewed in which the parents either were, or thought they were, sporadic cases three affected parents at the most have produced children, and only one of these, so far, has produced *affected* children.

The conclusion from this necessarily incomplete estimation of the mutation rate of the gene for retinoblastoma in Ohio is that it is much lower than that found by Neel and Falls in Michigan, and that this in turn is largely dependent upon the lower penetrance rate found existing among these Ohio families, which in turn may depend, in part at least, on the more intensive efforts expended in this study to trace antecedents as far as possible.

SUMMARY

A study of as complete a roster as possible of children born in Ohio in a 17 year period, and who developed retinoblastoma, was made. In 119 of 133 families, the disease appeared to be sporadic, despite intensive investigation into the family history. Hereditary cases (those in which sibs, parents or collateral lines were similarly affected) were significantly more often bilateral than were the sporadic cases. Bilateral cases have a much higher risk of dying with the disease. Only two instances of consanguinity were found. The degree of penetrance varies widely not only in different families but in different sibships in the same family. The families here presented indicate that the degree of penetrance may range as low as 20 per cent. The estimated mutation rate is of necessity most tentative, ranging for the total population from 1.76×10^{-5} to $.63 \times 10^{-5}$, depending upon whether all sporadic cases, or only bilateral sporadic cases are interpreted as germinal mutations. The rate for Negroes is higher than it is for the white race. The risk of sporadic cases producing affected children cannot be determined from the very small number of such cases in this study. The risk of normal parents producing more than one affected sib depends upon one's method in determining the risk. In this study, the ratio of families in which normal parents produced more than one affected sib to all families of normal parents producing any affected sibs was one in 15.

ACKNOWLEDGMENTS

1. This work was sponsored by a grant from the Department of Health, Education and Welfare, Field Investigations and Demonstrations Branch, from 1956 through May 1959.

2. The author desires to express her gratitude to those physicians and hospitals who so generously responded to requests for names of patients, and to the Director of the Columbus School for the Blind and to the Directress for the Ohio Services to the Blind for their co-operation.

REFERENCES

FALLS, H. F., AND NEEL, J. V. 1951. Genetics of retinoblastoma. *Arch. Ophth.* **46**: **367–389.**

FRANCESCHETTI, A., AND BISCHLER, V. 1946. Rétinoblastome et hérédité. *Arch. Julius Klaus Stift.* 21: 322.

NEEL, J. V., AND FALLS, H. F. 1951. The rate of mutation of the gene responsible for retinoblastoma in man. *Science* 114: 419–422.

REESE, A. B. 1949. Heredity and retinoblastoma. *Arch. Ophth.* **42**: **119–122.**

REESE, A. B. 1954. Frequency of retinoblastoma in the progeny of parents who have survived the disease. *Arch. Ophth.* **52**: **815–818.**

SOVIK, W. E. 1952. Bilateral retinoblastoma in six siblings. *Am. J. Ophth.* **35**: **1611–1618.**

TUCKER, D. P., STEINBERG, A. G., AND COGAN, D. G. 1957. Frequency of genetic transmission of sporadic retinoblastoma. *Arch. Ophth.* **57**: **532–535.**

VOGEL, F. 1954. Über Genetik und Mutationsrate des Retinoblastoms. *Zschr. menschl. Vererb.* **32**: **308–336.**

A Gm-like Factor Present in Negroes and Rare or Absent in Whites: Its Relation to Gm^a and Gm^x [1]

ARTHUR G. STEINBERG, BRENDA DAWN GILES, AND RACHEL STAUFFER

Department of Biology, Western Reserve University, Cleveland, Ohio

GRUBB (1956) FOUND that some human sera contain a factor capable of inhibiting the ability of serum from selected rheumatoid arthritic patients (RA serum) to cause Rh(+) cells coated with selected incomplete anti-D sera to agglutinate. Grubb and Laurell (1956) showed that this factor is contained in the gamma-globulin fraction of serum and therefore named it Gm^a. Individuals having the factor are called Gm(a+), those lacking it Gm(a−). Grubb and Laurell (1956) reported that 59.7 per cent of 360 Swedes and 94.6 per cent of 74 Eskimos were Gm(a+). They showed further that the presence of the factor was probably due to a dominant allele (Gm^a), its absence due to one or more undetected alleles collectively symbolized as *Gm*. The genetic observations were confirmed by Moullec, Kherumian, Sutton, and Espagnon (1956) and by Linnet-Jepsen, Galatius-Jensen, and Hauge (1958).

Moullec *et al.* (1956) reported that work in progress indicated that the newborn infant appeared always to have the same Gm group as its mother. Brønnestam and Nilsson (1957) confirmed this by a study of the Gm factor of 74 mothers and their newborn infants. The infant's Gm reaction was invariably identical with its mother's reaction. Linnet-Jepsen (cited in Linnet-Jepsen *et al.*) studied 165 mother-newborn infant pairs and followed 113 of the infants over a period of months. He reports that the Gm group of the infant does not yet seem to be fully developed at eight months. Grubb and Laurell (1956), Laurell and Grubb (1957), and Linnet-Jepsen *et al.* (1958) offered evidence to indicate that the Gm locus is independent of sex, the Hp groups, secretor, and the ABO, MNS, Rh, P, Lewis, Kell, Lutheran, and Duffy blood groups.

Population studies have been done on 360 Swedes (Grubb and Laurell, 1956), 871 Frenchmen (300 by Moullec *et al.*, 1956, and 571 by Podliachouk *et al.*, 1958), 1,084 Danes (Linnet-Jepsen *et al.*, 1958), 320 Norwegians (Harboe and Lundevall, 1959), and 74 Eskimos (Grubb and Laurell, 1956).

The frequency of Gm(a+) individuals among the European populations ranged from 54.3 (Moullec *et al.* on Frenchmen) to 60.6 (Harboe and Lundevall on Norwegians), but the differences are not significant. A total of 2,635 white individuals were tested in the five studies, and 1,501 (56.96 per cent) of them

Received August 17, 1959.

[1] Supported in part by Research Grant A-1931 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health.

were Gm(a+). This contrasts with 94.6 per cent Gm(a+) among 74 Eskimos (Grubb and Laurell, 1956).

Harboe and Lundevall (1959) reported a new factor (Gm^x) believed by them to be an allele of Gm^a, although they indicate that their data do not exclude a two-locus hypothesis. They found that 82 (25.8 per cent) of 318 Norwegians were positive for this factor and that it is inherited as a dominant. All Gm(x+) individuals were also Gm(a+); hence, only three phenotypes were observed in their population and in their family data, namely, Gm(a+ x+), Gm(a+ x−), and Gm(a− x−).

More recently Harboe (1959a and b) reported a system which tests for an allele of Gm^a whose frequency could account for the Gm(a−) class originally reported by Grubb and Laurell. This allele is called Gm^b (Harboe, 1959b).

In 1958 we decided to test an American Negro population to determine the frequency of Gm(a+) individuals among them. Accordingly, we attempted to find appropriate RA and anti-D sera to detect Gm^a. This paper is a report of our investigations.

MATERIALS AND METHODS

Sera from patients suffering from rheumatoid arthritis were kindly supplied by Dr. Robert Stecher and Dr. Paul Vignos. Anti-D sera were kindly supplied by Dr. Richard Rosenfield, Dr. Russell Weisman, Dr. Roger Marsters, Dr. Eloise Giblett, and Dr. F. H. Allen, Jr. We are grateful to these people for their generous cooperation.

The procedures used for testing were the same as those described by Linnet-Jepsen *et al.* (1958) except that quantities for the tests were measured in drops and, equating one drop to .05 ml., all quantities were halved in later tests. We found also that the Rh(+) cells could be used for at least six days and probably longer. Thirty-five RA sera and 25 anti-D sera were tested (not in all possible combinations, however) to find usable reagents.

Sera from unrelated white and Negro individuals were obtained from the routine hematology laboratory of the University Hospitals Out-Patient Department. Sera for family studies were obtained from families being investigated in other genetic studies in this laboratory.

THE DATA

We did not succeed in finding a pair of sera (RA-anti-D) giving the Gm^a reaction, but we did find relatively early in our testing an RA serum (Bomb.) from a white patient, which when used at a dilution of 1:20 in saline with an anti-D (Warren) from a Negro patient used at a dilution of 1:10 in saline gave an easily readable and reproducible reaction system.

This system has now been tested against 250 adult whites and against 403 adult Negroes with the results shown in Table 1. It is apparent that the factor is absent or rare among whites (P of observing none positive among 250 ~ .005 if the frequency is as high as .02; and ~.1 if the frequency is as low as about .01). It occurs with a frequency of .275 ± .022 among Negroes. Dr. Sylvia Lawler

TABLE 1. FREQUENCY OF THE GM-LIKE FACTOR AMONG NEGROES AND WHITES

	Total	Gm-like			
		+		−	
		No.	%	No.	%
Adult Negro population	265	73	27.5	192	72.5
Negro parents of the families studied	138	38	27.5	100	72.5
Total adult Negroes	403	111	27.5	292	72.5
Whites	250	0	—	250	100.0

TABLE 2. SUMMARY OF TESTS OF NEGRO FAMILIES FOR THE GM-LIKE FACTOR

Both Parents + Offspring					One Parent + Offspring					Neither Parent + Offspring			
s	r	n_{sr}	+	−	s	r	n_{sr}	+	−	s	r	n_{sr}	−
2	0	2	4	0	1	1	2	0	2	1	1	3	3
3	1	1	2	1	2	0	1	2	0	2	2	9	18
	2	1	1	2		1	5	5	5	3	3	5	15
5	0	1	5	0		2	3	0	6	4	4	7	28
6	0	1	6	0	3	1	4	8	4	5	5	4	20
						2	3	3	6	6	6	6	36
Total		6	18	3	4	2	3	6	6	7	7	2	14
					5	1	1	4	1	8	8	1	8
					6	2	1	4	2				
					7	4	1	3	4	Total		37	142
					8	4	2	8	8				
					Total		*26	43	44				

s = number in sibship.
r = number who are Gm-like negative.
* In 13 families the father was + and the mother −, and in 13 the mother was + and the father −.

of the Galton Laboratory kindly confirmed our testing for this factor by retesting 12 serum samples with our reagents.

Studies of Negro families were done to establish the pattern of inheritance. A summary of the data is presented in Table 2. We note in passing that the families were typed for secretor and the ABO, MN, Rh, Kell, Duffy, Jk, Js, Lu, and P blood groups. They were tested also for haptoglobins (Hp) and transferrins (Tf) by Dr. Eloise Giblett. If, on the basis of any of these tests or by the mother's admission, a child was shown to be extra-marital the family was not included. Nevertheless, it is possible that some families with extra-marital children have been included. The blood group, Hp, and Tf data will be reported at a later date.

The observed and expected numbers of matings in which both, one, or neither

TABLE 3. OBSERVED AND EXPECTED NUMBERS OF MATINGS IN WHICH BOTH, ONE, OR NEITHER PARENT IS GM-LIKE POSITIVE

Number of Parents +	Observed	Expected
2	6	5.2
1	26	27.5
0	37	36.2
Total	69	68.9

parent is positive are shown in Table 3. (The expected numbers were computed on the basis of 111 positive in a total of 403.) We may conclude that mating is random for this character.

ANALYSIS OF THE FAMILY DATA

The data in Table 2 indicate that when neither parent is positive for the Gm-like factor, none of the children is positive. The probability that 142 children would be negative by chance = $(.725)^{142}$ or $\sim 1.5 \times 10^{-10}$. This observation and the indication in Table 2 that more children are positive when both parents are positive than when only one parent is, suggests that the presence of the factor may be due to a dominant gene.

This may be tested (a) by computing the expected number of segregating (families with at least one recessive child) and of non-segregating families among those in which at least one parent is positive and (b) by estimating the proportion of recessives among the offspring of the segregating families and comparing the estimate with the expected proportion. The former test was done with the aid of the tables published by C. A. B. Smith (1956); the latter was done by the maximum likelihood method with the aid of the tables published by Finney (1949).

There were six matings in which both parents were positive and two of these were segregating (Table 2). The expected number is 3.06 (variance 1.4059). There were 26 matings in which only one parent was positive and 25 of these were segregating. The expected number is 19.89 (variance 4.2957). The difference is significant ($P \sim .017$); but the sample is small, and the deviation is in the opposite direction to that found for families in which both parents were positive. When both sets of matings are combined, the expected number of segregating families is 22.9 ± 2.39 and the observed 27. The difference is not significant ($P \sim .09$).

The maximum likelihood estimate of the proportion of recessive offspring in segregating families with one parent positive is $.43 \pm .06$; the expected value is .50, hence the difference is not significant.

We conclude that the Gm-like factor is due to a dominant gene. Its frequency in American Negroes is $.149 \pm .013$. It is absent or very rare in American whites.

The deficit of Gm-like(+) children from segregating matings with one parent positive (.43 rather than .50) cannot be explained by assuming that, as in Gm^a, children do not express their genotype until some time after birth. The age distribution of children with mothers positive is the same as that with

TABLE 4. TESTS OF SERA FROM NEGROES FOR GM-LIKE AND FOR GM^a

	Gm^a		
Gm-like	+	−	Total
+	28	0	28
−	68	2	70
Total	96	2	98

mothers negative, and direct observation shows that in the former case, 22 children were positive and 23 were negative, while in the latter, 21 were positive and 21 negative.

We have not yet tested newborn infants and their mothers, but we have observed an eight-month old Gm-like(+) infant whose mother was Gm-like(−). We have also observed several one- and two-year olds whose Gm-like type differed from their mothers'. We have not examined enough very young children to be able to evaluate the data quantitatively.

RELATION OF GM-LIKE FACTOR TO GM^a

Through the generosity of Dr. Sylvia Lawler, Dr. Hugh Fudenberg, and Dr. Morten Harboe, we obtained reagents to test for Gm^a.

Sera from 98 Negroes were tested for Gm-like and for Gm^a. Only two were Gm(a−) and both were also Gm-like(−) (Table 4). The probability that the distribution may have arisen by chance, assuming independence of Gm^a and Gm-like, is .51.

The expected frequency of Gm-like(+), Gm(a−), assuming they are independent, is approximately .0056 ($.02 \times .28$). Only about 2 in 1,000 matings may be expected to be between an individual heterozygous for Gm-like and Gm^a and an individual negative for both, again assuming independence of the two factors. Clearly, it will require an enormous sample or extraordinary good luck to determine the genetic relation between these two factors.

There seems little to be gained by outlining a series of possible relations between these two factors. The more likely ones are obvious, and none can be evaluated with our present data.

RELATION OF GM-LIKE TO GM^x

Dr. Morten Harboe kindly sent us samples of his reagents to test for Gm^x.

After establishing that we could duplicate his findings with the control sera he sent to us, we tested the sera of Negroes for Gm^x. During the course of this testing we found that an anti-D serum (M-18), of which we had only a small supply, and an RA serum (Bowers) duplicated the Gm^x test. With these reagents and those supplied by Dr. Harboe, we were able to test sera from 75 unrelated Negroes. The data are presented in Table 5. It is clear that Gm-like and Gm^x are not the same, and that Gm^x is much rarer in Negroes than in whites. As in the case of Gm^a, it well require a large sample to enable us to determine the genetic relation between these two factors.

TABLE 5. TESTS OF SERA FROM NEGROES FOR GM-LIKE AND FOR GM^{x}

	Gm^{x}		
Gm-like	+	−	Total
+	0	16	16
−	1	58	59
Total	1	74	75

SPECIFICITY OF THE REAGENTS

Grubb (1958) and Harboe (1959) demonstrated that as a rule only anti-D sera from individuals positive for a particular Gm factor can be used to test for that factor. Harboe (1959) reported that an anti-D serum from a Gm(a+ x+) individual could be used to test for Gm^{a} with one RA serum and for Gm^{x} with a different RA serum. Note that the specificity of the reaction is determined by the RA serum. We were able to confirm Dr. Harboe's findings using his reagents.

Experience with RA (Bomb.) demonstrates that the specificity of the reaction does not necessarily reside in the RA serum. Dr. Sylvia Lawler, to whom we sent RA (Bomb.) and anti-D (Warren) (the reagents used to test for Gm-like), found that RA (Bomb.) and her anti-D (1386) tested for Gm^{a}. Here then is an RA serum which can test for two factors, with the specificity being determined by the anti-D. We were able to confirm and to extend these observations with RA (Bomb.) and with RA (Bowers). A summary of our findings, which will be presented in detail elsewhere, is given in Table 6.

RA (Bowers) may be used to test for Gm^{a} and Gm^{x}, the specific factor being determined by the anti-D used. We found that RA (Bomb.) may be used not only to test for Gm^{a} and Gm-like, but also for Gm^{b}, depending upon the anti-D used. [The findings on RA (Bomb.) have been independently confirmed by Harboe (personal communication).] Thus we now have two RA sera which can detect more than one RA system, and with the specificity being determined by the anti-D serum rather than the RA serum.

In parallel with Harboe's findings we observed that the specificity for anti-D (Kimb.) and for anti-D (Ham.) in the Gm reaction is conferred by the RA serum used (Table 6). But note that anti-D (Kimb.), which can detect Gm^{a} or Gm^{b}, detects Gm^{b} with RA (Bomb.), although RA (Bomb.) can detect Gm^{a}

TABLE 6. RA-ANTI-D SYSTEMS USED TO TEST FOR VARIOUS GM FACTORS (The Gm factors are listed in the body of the table.)

Anti-D (Source)	RA (Bowers)	RA (Bomb.)
Kimb. (B.G.L.)	a	b
Ham. (B.G.L.)	x	b
1386 (Lawler)	—	a
And. (Giblett)	—	b
Warren	—	Gm-like

— = not tested.

when used with anti-D (1386) and with some other anti-D sera not listed in Table 6. Hence an anti-D serum which serves to detect Gm^a with one RA serum may not do so with another RA serum even though the latter may be used to detect Gm^a with a second anti-D (Table 6). Clearly, the specificity resides in the RA-anti-D system as such and not in either alone, although it does appear that the anti-D donor must have the factor for which the anti-D is used to test. It does not follow, however, that if the donor has the factor the anti-D is necessarily usable. It seems on the other hand that the donor of the RA serum need not have the factor being tested for. We say this because Mrs. Bomb., the donor of the RA used to test for Gm-like, is white. The reader will recall that none of the 250 whites was positive for Gm-like. Further discussion of the nature of the Gm reagents will be reserved until investigations now in progress have been completed.

Grubb (1958) reported that various methods of analysis show that the Gm^a factor is located in the gamma globulin fraction and that the inhibiting capacity of Gm(a+) sera varies with the gamma globulin concentration. This suggests that Gm(a+ x+) individuals may differ only quantitatively from Gm(a+ x−) individuals because no Gm(a− x+) individuals have been reported (Harboe and Lundevall, 1959). It suggests also that the high frequency of Gm(a+) among American Negroes may be due to the relatively higher concentration of gamma globulin in their serum. By extension of this argument it would appear that Negroes who are Gm-like(+) should have the highest gamma globulin content in their serum. The fact that none of the 16 Gm-like(+) Negroes who were tested for Gm^x was Gm(x+) and that the only Gm(x+) Negro was Gm-like(−) argues against merely quantitative differences among these factors. Further evidence that the differences among the factors are qualitative is derived from the data in Table 6. We refer to the observations that one RA serum may distinguish as many as three Gm factors and that one anti-D may distinguish at least two Gm factors.

SUMMARY

A new serum factor (Gm-like) detected by means of an RA serum-anti-D serum system similar to the one used to detect Gm^a is described. The factor was present in 27.5 per cent of 403 Negroes but in none of 250 whites. It is inherited as a dominant.

Only two of 98 Negroes tested for Gm^a were Gm(a−). Both were also Gm-like(−). We do not have data to evaluate the relation of Gm-like to Gm^a.

Only one of 75 Negroes tested was Gm(x+). He was Gm-like(−). Again, we do not have data to evaluate the relation between Gm-like and Gm^x beyond establishing that they are different.

Evidence is offered to show that the specificity of the reaction testing for the Gm factors lies in neither the RA nor the anti-D serum used, but in both as a reacting system.

REFERENCES

Brønnestam, R., and Nilsson, S. B. 1957. Gamma globulin groups (Gm) of mothers and their new-born infants. *Vox Sang.* 2 n.s.: 316–319.

FINNEY, D. J. 1949. The truncated binomial distribution. *Ann. Eugen.* 14: 319–328.

GRUBB, R. 1956. Agglutination of erythrocytes coated with incomplete anti-Rh by certain rheumatoid arthritic sera: the existence of other serum groups. *Acta path. microb. scand.* 39: 195–197.

GRUBB, R. 1958. Interactions between rheumatoid arthritic sera and human gamma globulin. *Acta haemat.* 20: 246–252.

GRUBB, R., AND LAURELL, A. B. 1956. Hereditary serological human serum groups. *Acta path. microb. scand.* 39: 390–398.

HARBOE, M. 1959a. A new haemagglutinating substance in the Gm system, anti-Gm. *Nature* 183: 1468–1469.

HARBOE, M. 1959b. A new hemagglutinating substance in the Gm system, anti-Gm^b. *Acta path. microb. scand.* 47: 191–198.

HARBOE, M., AND LUNDEVALL, J. 1959. A new type in the Gm system. *Acta path. microb. scand.* 45: 357–370.

LAURELL, A. B., AND GRUBB, R. 1957. The Hp and Gm groups and secretor characters of 46 blood donors. *Vox Sang.* 2 n.s.: 312–316.

LINNET-JEPSEN, P., GALATIUS-JENSEN, F., AND HAUGE, M. 1958. On the inheritance of the Gm serum group. *Acta genet. et stat. med.* 8: 164–196.

MOULLEC, J., KHERUMIAN, R., SUTTON, E., AND ESPAGNON, P. 1956. Contribution a l'étude du facteur de groupe Gm^a du plasma humaine. *Rev. hémat.* 11: 512–517.

PODLIACHOUK, L., JAQUELINE, F., AND EYQUEM, A. 1958. Le facteur serique Gm^a au cours des rhumatismes inflammatoires chroniques. *Ann. Inst. Pasteur* 94: 590–597.

SMITH, C. A. B. 1956. A test for segregation ratios in family data. *Ann. Eugen.* 20: 257–265.

Serum Enzymes and Genetic Carriers in Muscular Dystrophy

C. S. CHUNG, N. E. MORTON, AND H. A. PETERS

Departments of Medical Genetics and Neurology, University of Wisconsin

STUDIES FROM THIS LABORATORY have demonstrated that muscular dystrophy in man, exclusive of myotonic, myositic, neurogenic, ocular, and distal types, can be divided by genetic and clinical criteria into four categories: the dominant facioscapulohumeral type, the sex-linked Duchenne type, the recessive limb-girdle type, and the sporadic limb-girdle type (Chung and Morton, 1959; Morton and Chung, 1959). The first three classes had been recognized by other workers (Stevenson, 1953; Walton, 1955), but unambiguous proof of the fourth type required methods which have only recently become available (Morton, 1959). The correctness of this classification was established by analysis not only of our Wisconsin material, but also of seven other large studies from the literature which had been interpreted differently. The eight sources proved to be consistent with each other and with genetic expectation.

Schapira, Joly and Schapira (1953) and Dreyfus and Schapira (1955) showed that many cases of muscular dystrophy manifest abnormally high activities of several serum enzymes, notably aldolase, phosphohexoisomerase, and transaminase, presumably released from dystrophic muscle. Subsequent studies have indicated that serum levels of these enzymes are highest in rapidly evolving myopathies, in which they reach maximum values near the time of clinical onset and perhaps preclinically (Dreyfus, Schapira, Schapira, and Demos, 1956; Pearson, 1957). Normal values are usually found in neurogenic atrophies, including amyotrophic lateral sclerosis, amyotonia congenita, Charcot-Marie-Tooth disease, and poliomyelitis. Support for the muscular origin of elevated serum aldolase in myopathies comes from the observation of Zierler (1958) that excised peroneus longus muscle of dystrophic mice has a greater aldolase efflux than normal muscle, and the report of Fanny Schapira, Dreyfus and Schapira (1957a) that the relative activity of serum aldolase in dystrophic patients and in mice on fructose-1-phosphate and fructose-1,6-diphosphate is characteristic of muscle rather than liver aldolase. Elevation of serum enzyme levels is also observed in nutritional myopathy in the rabbit (Evans and Baker, 1957) and in acute myositis, myocardial infarction, acute hepatitis, and other diseases with rapid tissue destruction (Sibley and Fleisher, 1954).

Autosomal recessive muscular dystrophy has been studied in the mouse

Received Nov. 12, 1959.

Genetics paper number 752. This work was supported by grants from the National Institutes of Health, Muscular Dystrophy Associations of America, and the Graduate School of the University of Wisconsin.

(Michelson, Russel and Harman, 1955) and the chicken (Asmundson and Julian, 1956). These dystrophic strains promise to be of great value in research on human dystrophy, to which they appear to be essentially similar. They can be used to screen proposed treatments, to search for the biochemical bases of dystrophy, and to elucidate other questions which cannot be so easily approached in man, including the following: (1) Are serum enzymes of diagnostic value for preclinical cases, and (2) can heterozygous carriers be recognized.

METHODS

Serum glutamic-oxalacetic transaminase was assayed by the method of Karmen (1955), as modified by Steinberg, Baldwin and Ostrow (1956). One unit of transaminase is defined as the amount of enzyme that will cause the spectrophotometric reading at 340 mμ to decrease at the rate of .001 optical density units per minute per centimeter light path at 25° C. under the described conditions. The temperature during the assay was maintained at 37° C., and the results adjusted to 25° C. Creatine and creatinine in urine were determined by the method of Clark and Thompson (1949), using the Jaffe reaction, and were expressed as milligrams of creatinine per 24-hr. urine collection. Serum aldolase was assayed by an adaptation of the method of Sibley and Lehninger (1949), modified by Lowry, Roberts, Wu, Hixon and Crawford (1954). All readings were made in a Beckman DU spectrophotometer.

The procedure for the aldolase assay was as follows:

1. To a 10 ml. sample tube add 0.5 ml. of cold serum and 1.0 ml. of an ice-cold, fresh mixture of equal parts of 0.02 M magnesium fructose-1,6-diphosphate and 0.12 M hydrazine of pH 8.6. Mix without warming and place in a water bath at 37° C. For a control tube, omit serum.
2. Exactly 60 min. later, replace rack in ice water and to each tube add 0.3 ml. of 30% trichloroacetic acid, with thorough mixing by buzzing. To the control tube, add 0.5 ml. of serum.
3. Place 0.2 ml. of supernatant into a 10 ml. tube with 0.2 ml. of 0.6 N NaOH, tapping gently to mix.
4. After 30 min. at room temperature, add 0.3 ml. of 0.1% 2,4-dinitrophenylhydrazine in 2 N HCl, mixing carefully.
5. After 30 min. at room temperature, add 6 ml. of 1 volume of 1 N NaOH in 2 volumes of methyl Cellosolve, with thorough mixing by buzzing.
6. After another 30 min., read at 570 mμ.

The procedures of Lowry et al. (1954) were followed in preparing and handling the reagents, and in standardizing the results with methylglyoxal from dihydroxyacetone. Under these conditions, if x is the optical density, 2x is the aldolase activity expressed in Meyerhoff units as micrograms of alkali-labile phosphorus produced per minute per ml. of serum at 37° C. Serum with high aldolase activity was diluted with distilled H_2O and the results converted to Meyerhoff units per ml. of serum.

Functional ability of muscle was measured in chickens by the exhaustion number, defined as the number of times a bird can be placed on its back in

rapid succession, up to and including the first failure to get to its feet. Breast width in mm. was determined with soldering wire, fitted to the breast and then transferred to lined paper, as described by Asmundson and Julian (1956). Functional ability in patients was measured by the rating scale of Swinyard, Deaver, and Greenspan (1957) which ranks the first stage of dystrophy as condition 1 and the terminal stage as condition 8.

MUSCULAR DYSTROPHY IN CHICKENS

Eggs were obtained from Dr. V. S. Asmundson of his inbred New Hampshire Strain 3, which is homozygous for *am*, an autosomal recessive gene which produces pseudohypertrophic muscular dystrophy (Asmundson and Julian, 1956). Lacking an isogenic control, we crossed these birds to a local White Leghorn strain, using pooled semen from five White Leghorn males, and then intercrossed and testcrossed the F_1 progeny. Concurrently, the five original White Leghorn males were crossed to a local New Hampshire strain and this F_1 backcrossed to New Hampshires. From these matings we obtained progeny with several levels of heterosis and of different degrees of New Hampshire and Strain 3 ancestry, permitting exclusion of these extraneous effects from the comparison of +/+ controls, +/am carriers, and am/am dystrophics.

Aldolase and transaminase were converted to common logarithms to normalize the distributions and stabilize the variances for the three genotypes. We have found the logarithmic transformation generally helpful for biochemical assays, the standard deviations of which tend to be proportional to the means. To detect seasonal variations, the sine and cosine of month in radians were incorporated into the analysis. Heterosis was defined as 0 for intrastrain matings, ½ for F_2's and backcrosses, and 1 for F_1's. The proportion of New Hampshire ancestry was denoted by N. H., which takes the value 1 for purebred New Hampshires, ½ for the F_1 and F_2 with White Leghorns, and ¾ for the backcross of the F_1 to New Hampshires. The proportion of Strain 3 ancestry was denoted by N3, which takes the value 1 for purebred Strain 3's, ½ for the F_1 or F_2 outcross, and ¾ for the backcross of the F_1 to Strain 3.

The analysis of these variates for carriers vs. normals is shown in Table 1. With age in days after hatching, there is a highly significant decrease of aldolase and increase of exhaustion number and breast width. All three variables show seasonal variation when adjusted to the mean age of 140 days, exhaustion number being greatest in January and least in July, with an amplitude of 0.93. Aldolase is highest in the spring and lowest in the fall, with an amplitude of 0.145 log units, while breast width shows the opposite trend, with an amplitude of 1.83 mm. The reason for the aldolase cycle is unknown, but it is likely that the heat of summer is a factor in lowering the exhaustion number, while the drafts of winter may reduce growth rate through heat loss and respiratory disease. The birds were housed in quarters with poor temperature control. Transaminase is scarcely affected by age, and the seasonal cycle is much less marked, with an amplitude of 0.019 log units.

The most interesting feature of this analysis is the evidence for increased

TABLE 1. ANALYSIS OF VARIANCE OF CARRIER VS. NORMAL CHICKENS WITHIN SEX

Source	D. F.	Dependent variate mean square			
		Aldolase	Trans-aminase	Breast Width	Exhaustion No.
Age, age squared, month[1]	3	2.35**	.085	1843.99**	437.93**
Genotype after fitting above	1	1.82**	.051*	39.27*	35.87
Heterosis, N. H., and N3 after fitting above	3	.06	.019	2.35	15.36
Among birds	86	.05	.013	8.63	39.64
Replicates within birds	266	.09	.015	9.25	20.37

[1] The sine of month in radians was used for aldolase, transaminase, and breast width, and the cosine of month for the exhaustion number, the effect of the alternate function being nonsignificant.

* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

TABLE 2. MEANS ADJUSTED FOR AGE AND MONTH WITHIN SEX

	Log Aldolase	Log Transaminase	Breast Width
Carrier	0.285	2.323	28.55
Normal	0.132	2.296	27.46
Difference	.154 ± .025	.0264 ± .0140	1.08 ± 0.31

aldolase, transaminase, and breast width in carriers. Table 2 shows the means, the difference between carriers and normals being highly significant for aldolase (Figure 1), significant for breast width, and barely significant for transaminase. There is no significant difference in the exhaustion number. Apparently the dominance of the normal allele is incomplete, the heterozygote having a minimal error of metabolism which is usually not sufficient to produce any appreciable functional disability. Expression of dystrophic symptoms by a heterozygote must certainly be rare, but the increased enzyme levels and breast width of carriers suggest that heterozygotes may be more susceptible to myopathy than normal homozygotes. There is no significant effect of sex on the difference between carriers and normals. Previously Asmundson and Julian (1956) reported that carriers have wider breasts than homozygous normal birds of the New Hampshire breed. Our analysis tends to confirm their conclusion that this difference is not due to heterosis when Strain 3 is outcrossed, since effects of heterosis, New Hampshire ancestry, and Strain 3 ancestry on breast width are not significant.

Dystrophic and non-dystrophic birds are compared in Figure 2, which is based on multiple regressions. The dystrophics usually show abnormalities in serum aldolase, transaminase, and exhaustion number shortly after hatching. Hyperaldolasemia and transaminasemia are greatest, not preclinically, but after 150 days, at which time all dystrophics are unable to get up from their backs.

"Preclinical" dystrophic birds which were still able to get up were compared with young normals, adjusting for age. Judged from the sums of squares due to

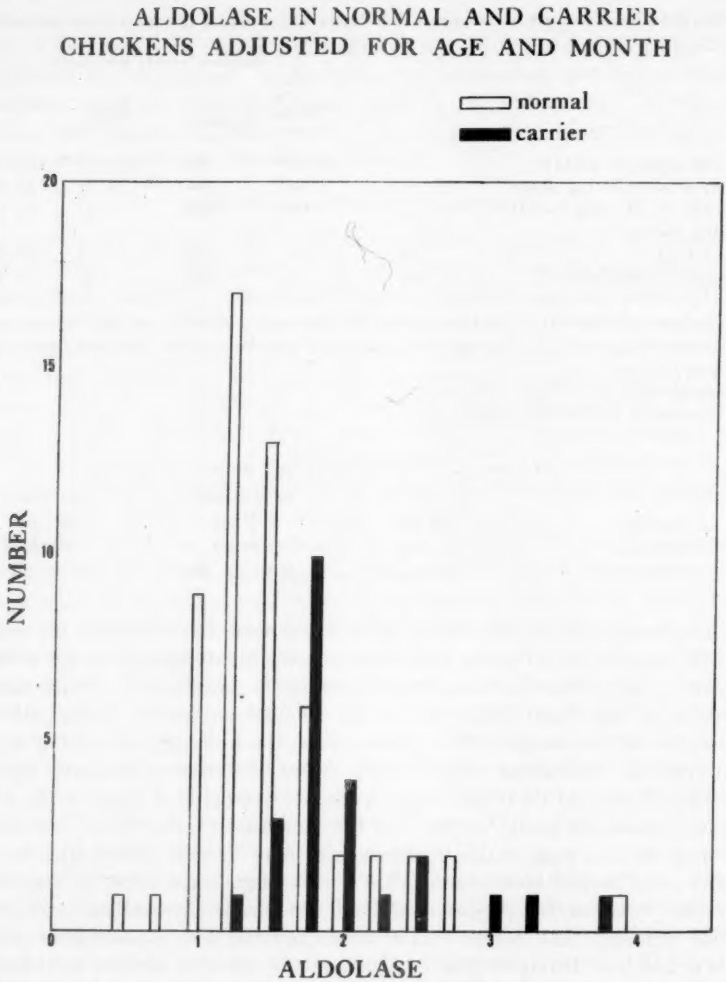

FIG. 1

regression of genotype on exhaustion number, breast width, transaminase, and aldolase, exhaustion number is the best discriminant of the preclinical state. However, the sum of squares due to partial regressions on aldolase, transaminase, and breast width are highly significant, indicating that serum enzyme levels and pseudohypertrophy are important adjuncts to muscle function tests in recognition of early cases of dystrophy. Aldolase accounts for a smaller sum of squares than transaminase, perhaps because normal hyperaldolasemia in young chicks obscures the effects of dystrophy. When aldolase and trans-

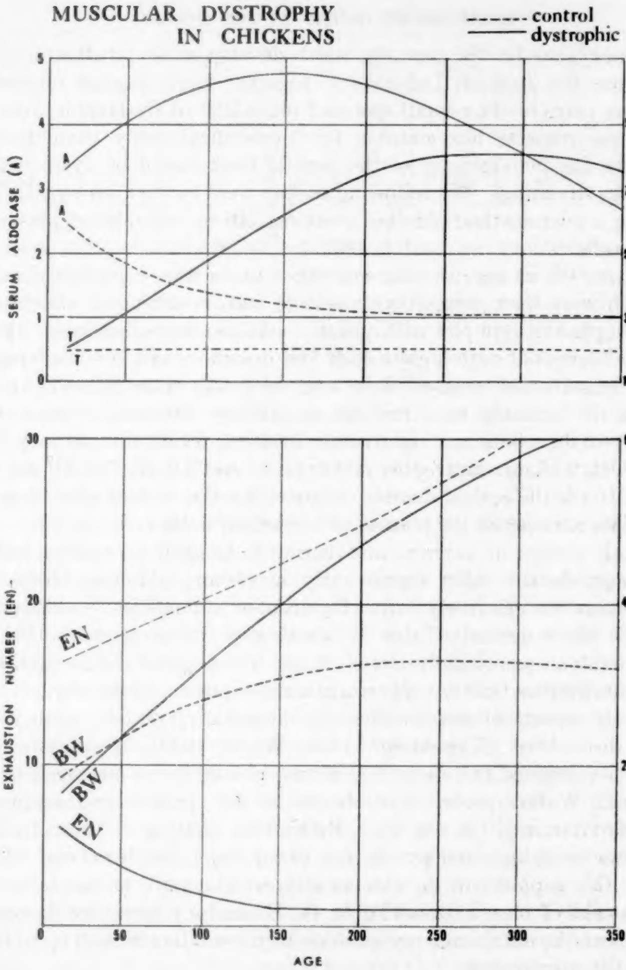

FIG. 2

aminase are used jointly to discriminate between dystrophic and non-dystrophic birds, the sum of squares due to transaminase is much greater than the contribution of aldolase. Evidently transaminase is more valuable than aldolase in the diagnosis of dystrophy in chickens, but since both partial regressions are highly significant, the two assays in conjunction are more useful than either one separately.

Dystrophic birds show exactly the same seasonal cycle in serum aldolase as normals, the amplitude being 0.145 log units.

MUSCULAR DYSTROPHY IN THE MOUSE

Mice homozygous for the gene *dy*, which develop muscular dystrophy, were received from the Jackson Laboratory, together with isogenic normals and heterozygous carriers. The small size and infertility of dystrophic mice make them in these respects less suitable for biochemical study than dystrophic chickens, but the homogeneity of the genetic background of dystrophics and controls is an advantage. The following studies were carried out by Mr. Dennis Olson, using a micromethod aldolase assay on .004 ml. samples of plasma from the caudal vein.

(Blood from the caudal incision was taken up in fine, heparinized capillary tubes, which were then plugged with sealing wax, centrifuged, and broken to separate the plasma. Samples with visible hemolysis were discarded. The clear plasma was blown out onto a glass slide and drawn up into a 4 λ micropipette. The same reagents and protocol were used as in the standard aldolase assay, except that the amounts were reduced as follows: 0.004 ml. plasma, 0.1 ml. fructose-1,6-diphosphate and hydrazine, 0.05 ml. TCA, 0.1 ml. supernatant, 0.1 ml. NaOH, 0.15 ml. dinitrophenylhydrazine, and 3.0 ml. NaOH and methyl Cellosolve. If x is the optical density, adjusted for the control tube, then 21.39x is the aldolase activity of the plasma in Meyerhoff units.)

Two small groups of carriers and normals, assayed at approximately 10 weeks of age, do not differ significantly in plasma aldolase. However, the standard errors are relatively large. Dystrophic animals have aldolase levels roughly five times normal (Table 3). Previously Schapira et al. (1957a) reported hyperaldolasemia in dystrophic mice, and pointed out that this is one of several similarities between this condition and human dystrophy.

Because of reports of improvement in muscular dystrophy with a special diet, now discredited (Van Meter, 1953; White, 1959), dystrophic mice at weaning were assigned two diets, one a standard 20% protein meal, the other enriched with Walker protein hydrolysate to 30% protein and supplemented further with vitamin E (50 mg./kg.), Rubrafolin (250 μg. vitamin B_{12}/kg. and 16.7 mg. folic acid/kg.) and pyridoxine (4 mg./kg.). No beneficial effect was exerted by this supplement on aldolasemia, weight gain, or mortality during the eight weeks of observation (Table 4). Hereditary muscular dystrophy in the mouse is unlike nutritional myopathies, which can be reversed up to terminal stages by diet supplements.

MUSCULAR DYSTROPHY IN MAN

Through a Muscular Dystrophy Clinic sponsored by the Muscular Dystrophy Associations of America, we were able to collect clinical, genetic and labora-

TABLE 3. ALDOLASE VALUES IN THE MOUSE

Gentoype	n	Aldolase units ± S. E.
Normal (+/+)	15	1.46 ± 0.27
Carrier (dy/+)	19	1.26 ± 0.24
Dystrophic (dy/dy)	22	6.56 ± 0.72

TABLE 4. DIET EFFECTS IN DYSTROPHIC MICE

Diet	Aldolase (U)		Wt. Gain (gm.)		Mortality	
	n	$\bar{x} \pm$ S. E.	n	$\bar{x} \pm$ S.E.	n	%
Control	7	4.94 ± 0.89	11	2.25 ± 0.36	12	67
Supplement	15	7.32 ± 0.93	11	2.65 ± 0.46	11	64

tory data on 109 patients with an established diagnosis of muscular dystrophy. In addition, 50 cases with other neuromuscular disease or deferred diagnosis were studied. Of the dystrophics, 2 were classified on genetic and clinical evidence as facioscapulohumeral, 41 as limb-girdle, and 66 as Duchenne, according to the discrimination procedure of Chung and Morton (1959), who estimated that there are about 204 manifest cases of these types of muscular dystrophy in Wisconsin. Of the limb-girdle cases, 28 could be classified by genetic evidence (group 2), but 13 were classified on clinical evidence alone (group 4[2]). Of the Duchenne cases, 11 could be classified by genetic evidence (group 3), but 55 were classified on clinical evidence alone (group 4[3]). For the 41 cases classified on genetic evidence as facioscapulohumeral, limb-girdle, or Duchenne, the clinical data were invariably consistent.

We did not have in this material any example of pseudohypertrophic limb-girdle dystrophy, which is clinically almost indistinguishable from the sex-linked Duchenne type. However, through the cooperation of the Hawaii Bureau of Crippled Children, one of us (N. E. M.) had an opportunity to study a family with pseudohypertrophic limb-girdle dystrophy in three siblings, two boys and a girl, on the island of Hawaii. They have been included with the Wisconsin limb-girdle cases for purposes of comparison.

For aldolase and transaminase controls* we had 114 normal volunteers, including laboratory workers, husbands and wives of patients, and fathers and older brothers of Duchenne cases. A small group of hospital "controls" was discarded because it was significantly higher than normals in creatine and aldolase and lower in transaminase, after adjustment for age. There was no significant effect of sex on transaminase and aldolase. Figure 3 shows the normal range which is delimited by two broken lines above which 2.5 per cent of normals are expected to lie. At age 30 the mean is 0.214 U, with a range of 0.112 to 0.406

* It was necessary to modify the aldolase assay for infants and advanced dystrophics who did not lend themselves to venepuncture. Blood from a finger prick was taken up in several capillary tubes, which were then plugged with sealing wax, centrifuged, and broken to separate the serum. Samples with visible hemolysis were discarded. The clear serum was blown out into a tapered centrifuge tube, and equal amounts were drawn up into two 0.1 ml. pipettes, one for the assay and one for the blank. Amounts of reagents were reduced to 0.5 ml. fructose-1,6-diphosphate and hydrazine, 0.15 ml. TCA, 0.1 ml. supernatant, 0.1 ml. NaOH, 0.15 ml. dinitrophenylhydrazine, and 3.0 ml. NaOH in methyl Cellosolve. If y ml. of serum is used in the assay tube, the optical density corrected for the blank should be multiplied by 10(.65 + y)/18y, to give the aldolase activity in Meyerhoff units. This capillary method was checked against the standard assay and found to give comparable results.

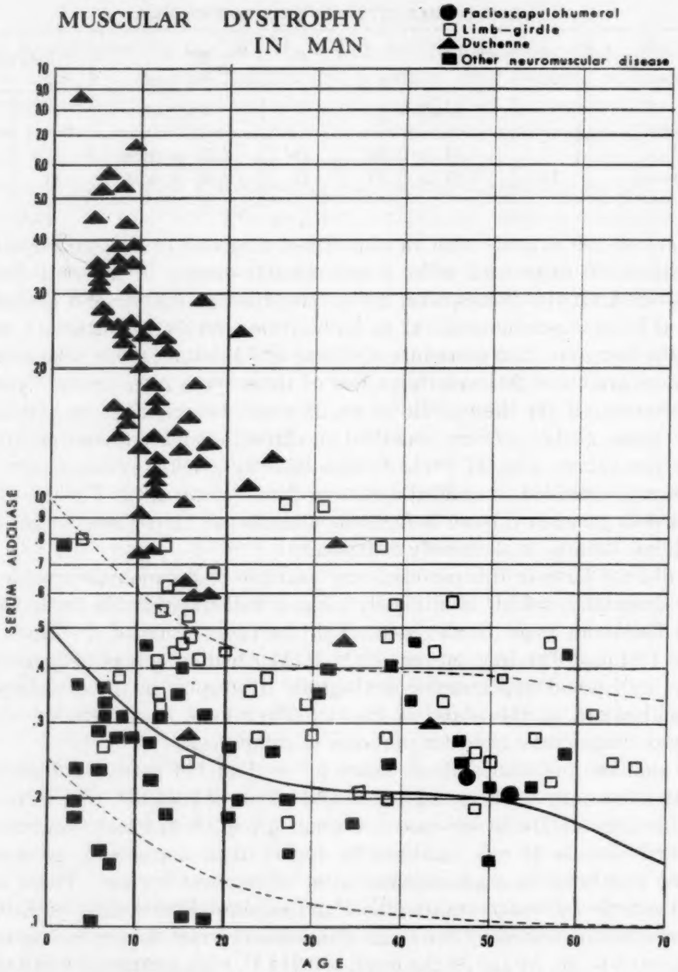

FIG. 3

U, in good agreement with other reports on adults (Sibley and Lehninger, 1949; Schapira et al., 1955; Bruns, 1954). At age 10 the mean is 0.310 U, with a range of 0.161 to 0.592 U, which compares well with children reported by Schapira et al. (1955).

On the same figure are charted the dystrophic patients and other cases of neuromuscular disease. Of 66 Duchenne cases, all but 2 are above the normal limit, these exceptions being far advanced cases. The elevation is most marked in young patients, in whom the disease has been of short duration. One younger

sibling, who developed dystrophy during the study, was found to have pronounced hyperaldolasemia (8.50 U) at age 3 when parental concern was first aroused. Pseudohypertrophy was questionable. The disability in this boy was then very slight, but he is now a typical Duchenne case. Another boy, also the younger sibling of a proband, was suspected by his parents of muscle weakness at age 2. Clinical examination confirmed the impression of slight physical retardation, but did not establish a diagnosis of dystrophy. Aldolase and transaminase were normal. This child is now developing normally. Other observers (Dreyfus et al., 1956; Pearson, 1957; and Murphy and Cherniak, 1958) report similar findings, which testify to the value of serum enzyme tests in the diagnosis of early dystrophy, especially of the Duchenne type.

Of the 43 limb-girdle cases, nearly all were above the normal mean, but only 14 were above the upper limit of normal. These include the younger brother of an affected girl, who was observed when he visited the clinic with his sister at age 14 to have a very slight disability, which the parents professed not to have noticed. His aldolase level was 0.55 U. This boy now has developed unmistakable dystrophy, showing that the usefulness of serum enzyme tests in establishing the diagnosis of muscular dystrophy extends to the limb-girdle type.

Both facioscapulohumeral cases and all but 4 of the 50 cases of other neuromuscular disease had aldolase levels below the upper limit of normal. These comprised 17 cases of Oppenheim's disease, 12 of Charcot-Marie-Tooth disease, 3 of convalescent myositis, 1 of myotonia congenita, 5 of myotonia dystrophica, 1 of pyramidal atrophy, and 11 diagnoses deferred. The elevated values occurred in 2 patients with myotonia dystrophica, in 1 deferred diagnosis, and in 1 patient considered to represent a mixed myopathy and Charcot-Marie-Tooth disease, with myopathic electromyogram, neuropathic biopsy, and fibrillations. No case considered on clinical evidence to be a pure neuropathy showed hyperaldolasemia.

Seven cases (2 convalescent myositis, 3 Oppenheim, 1 Charcot-Marie-Tooth disease, and 1 diagnosis deferred) had aldolase levels below the lower limit of normal. If serum aldolase reflects the rate of muscle destruction, it is not surprising that some atrophies with reduced muscle mass, but no active wasting, should have hypoaldolasemia.

Figure 4 shows the pronounced association between disability and aldolase. Within each condition, the facioscapulohumeral, limb-girdle, and Duchenne cases are clearly distinguishable. Within the limb-girdle class, there was no significant difference between isolated and familial cases or between males and females. Enzyme levels tended to increase during the winter and decrease during the summer, but this difference was not statistically significant. Schapira et al. (1957b) reported seasonal aldolase changes in French dystrophics. The relevance of this to dystrophy is doubtful, since normals show the same variation, as do normal chickens. The amplitude is .035 log units for normals, .026 for limb-girdle cases, and .038 for Duchenne cases.

Transaminase assays were carried out on the majority of patients. In contrast to avian dystrophy, affected individuals are discriminated very much better by

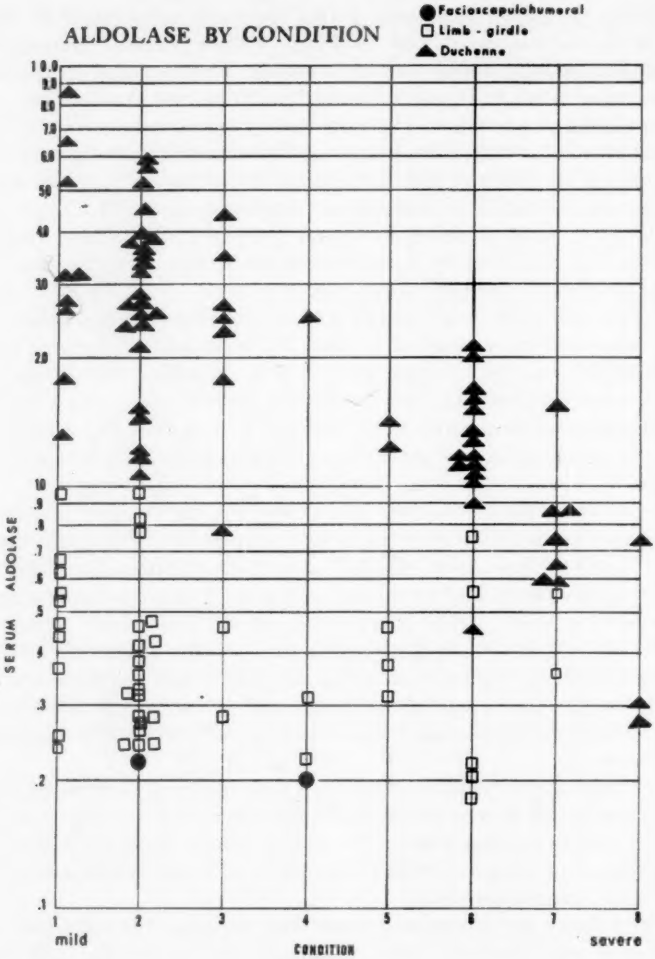

FIG. 4

aldolase than by transaminase, the sum of squares due to partial regression of affection on transaminase being nonsignificant, while the partial regression on aldolase is very highly significant.

Previously we found that the inbreeding coefficient, pseudohypertrophy, and contractures accounted for all of the significant clinical discrimination between Duchenne and limb-girdle cases (Chung and Morton, 1959). When this information was combined with aldolase and transaminase, the discrimination afforded by partial regression on aldolase was highly significant, but the partial regression on transaminase was nonsignificant. This indicates that aldolasemia

is complementary to clinical evidence in the resolution of limb-girdle and Duchenne dystrophy, and not merely a correlate of pseudohypertrophy, while the transaminase assay adds no information to the aldolase test. The best discriminant is

$$D = -8.6100F + .4920P + .2424C + .4397A,$$

where F is the inbreeding coefficient, P the presence of pseudohypertrophy, C the presence of contractures, and A the log aldolase level. The values of D range from .98 to 1.86 for limb-girdle cases and 1.80 to 2.41 for Duchenne cases.

The only overlap is for the two cases of pseudohypertrophic limb-girdle dystrophy whose aldolase levels were determined. They are represented in Figure 4 by the two lowest values for condition 5. At their stage of the disease, at ages 23 and 29, aldolasemia and contractures are less pronounced than in Duchenne cases with the same disability. So far as these observations go, they support the contention that some pseudohypertrophic limb-girdle cases are clinically distinct from Duchenne cases (Stevenson, 1953; Becker, 1953). However, other case histories of the two genetic types appear to be indistinguishable (de Grouchy, 1953; Walton, 1955). Very high aldolase levels have been observed in some girls with pseudohypertrophic dystrophy (Schapira et al., 1957b), indicating that aldolasemia is not pathognomonic of sex-linkage. However, in our material and probably in the great majority of cases, the aldolase level is as good a discriminant of this genetic type as pseudohypertrophy, and is more objective.

Within the limb-girdle and Duchenne classes there is a marked clinical resemblance of affected relatives (Chung and Morton, 1959), which is not increased by taking aldolase into account.

Within the limb-girdle and Duchenne classes there was no marked association between the aldolase level and the estimated rate of progression of dystrophy, based on two or more muscle charts. Of course, if the limb-girdle and Duchenne types are pooled, there is a striking association between aldolase level and rapid progression.

When normal controls are compared with unaffected relatives of dystrophics, adjusting for month and age, the carriers of the sex-linked gene for Duchenne dystrophy have significantly elevated aldolase levels, the mean logarithmic difference being $.073 \pm .035$. Other Duchenne relatives not known to be carriers differ in the same direction, but not significantly, the difference being $.042 \pm .026$. Known limb-girdle carriers, and other relatives, are not significantly different from the control (Table 5).

Several female relatives of Duchenne cases had aldolase levels far above normal limits. One was the 10 year old sister of an isolated Duchenne boy. On four occasions, she had aldolase levels of 1.80, 1.15, 1.19, and 1.40, the average being 4.47 standard deviations above the normal mean for her age. The probability of such a deviation in a normal population is less than .00001. Clinical and laboratory studies showed no other abnormality. Another deviant was the 13 year old sister of an isolated Duchenne boy. She had an aldolase level

TABLE 5. SERUM ALDOLASE BY GENOTYPE, ADJUSTED FOR AGE AND MONTH

Source	Mean (U)	Elevated Values*
Control	.228	1/131
Known carrier, limb-girdle	.221	1/19
Known carrier, Duchenne	.269	3/21
Other relative, limb-girdle	.203	0/44
Other relative, Duchenne	.251	6/72

* A value is considered elevated if, after adjustment for age and month, it exceeds .509 U, which in logarithms is 1.96 standard deviations above the control mean.

of 1.023, approximately 3.8 standard deviations above the mean for her age ($P < .001$). Seven other female relatives, three of them known carriers, exceed the normal limits for their age, with no other apparent abnormality (Table 5). We postulate that a minority of carriers of the gene have aldolasemia, but are otherwise asymptomatic. The possibility that the two highest levels for Duchenne dystrophy are preclinical cases is not ruled out, although the fact that both are females speaks against this.

It should be emphasized that serum aldolase does not provide a reliable test for carriers, since only a small proportion of heterozygotes exceed the normal limit. Nevertheless, if a secondary symptom like aldolasemia allows us to recognize some carriers, it may confidently be predicted that the discovery of the fundamental metabolic error will permit accurate carrier detection.

One carrier mother of two Duchenne boys was found to manifest typical symptoms of mild limb-girdle dystrophy without aldolasemia at age 50. She considered herself normal until age 42, when she began to have weakness in the legs, later extending to the arms. She cannot wear high heels or touch the floor, and has difficulty with stairs. Her left arm is wasted, the right much less so. Her creatine/creatinine ratio was .65 on one occasion, .73 on another, indicating considerable muscle wasting. She was diagnosed clinically as limb-girdle dystrophy, although not so severely affected as to seek medical attention for herself. Kryschowa and Abowjan (1934) reported dysplastic symptoms in some female carriers of the gene for Duchenne dystrophy, including kyphosis, enlarged calves, and facial weakness. These observations, especially our case of verified dystrophy, lend support to the suggestion that some cases of sporadic limb-girdle dystrophy are carriers in whom unknown factors have brought a usually recessive gene to expression.

Morton and Chung (1959) showed that the frequency of sporadic limb-girdle dystrophy is 27 persons who will develop the disease per million births, and the frequency of carriers of recessive limb-girdle dystrophy is 16 per thousand births. Therefore, if only 2 per thousand carriers were affected, this would account for all sporadic limb-girdle cases.

SUMMARY

Elevation of serum enzymes is characteristic of preclinical dystrophics in chickens, and probably in man. At the time the condition is first diagnosed, serum levels are characteristically elevated, especially in the rapidly evolving

sex-linked type. Heterozygous carriers have increased enzymes and breast width in chickens, increased aldolase in a minority of humans, and physical disability and creatinuria in a still smaller proportion.

ACKNOWLEDGMENT

The authors wish to acknowledge the help given them by Mrs. Hannelore Lennig and the staff of the Muscular Dystrophy Clinic in studying these families, by the Wisconsin State Medical Society and the Bureau for Handicapped Children in finding cases, and by the patients and their families whose cooperation made this study possible. The dystrophic chickens were kindly supplied by Dr. V. S. Asmundson and maintained with the advice and assistance of Dr. W. H. McGibbon. Mr. Dennis Olson carried out the studies on dystrophic mice, during the tenure of a medical student research fellowship.

REFERENCES

Asmundson, V. S., and L. M. Julian. 1956. Inherited muscle abnormality in the domestic fowl. *J. Hered.* 47: 248–252.

Becker, P. E. 1953. *Dystrophia musculorum progressiva. Eine genetische und klinische Untersuchung der Muskeldystrophien.* Sammlung Psychiatrischer und Neurogischer Einzeldarstellungen. Stuttgart.

Bruns, G. 1954. Bestimmung und Eigenschaften der Serumaldolase. *Biochem. Zschr.* 325: 156–162.

Chung, C. S., and N. E. Morton. 1959. Discrimination of genetic entities in muscular dystrophy. *Am. J. Human Genet.* 11: 339–359.

Clark, L. C., and H. L. Thompson. 1949. Determination of creatine and creatinine in urine. *Anal. Chem.* 21: 1218–1221.

De Grouchy, J. 1953. *Contribution a l'Étude de l'Hérédité de la Myopathie.* Thesis: Paris.

Dreyfus, J.-C., and G. Schapira. 1955. L'activité transaminasique du sérum au cours des myopathies. *C. rend. Soc. biol.* 149: 1934–1935.

Dreyfus, J.-C., G. Schapira, Fanny Schapira, and J. Demos. 1956. Activités enzymatiques du muscle humain. *Clin. Chim. Acta* 1: 434–449.

Evans, J. H., and R. W. R. Baker. 1957. Serum aldolase and the diagnosis of myopathy. *Brain* 80: 557–570.

Karmen, A. 1955. A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum. *J. Clin. Invest.* 34: 131–133.

Kryschowa, N., and W. Abowjan. 1934. Zur frage der Heredität der Pseudohypertrophie Duchenne. *Zschr. ges. Neur. Psychiat.* 150: 421–426.

Lowry, O. H., N. R. Roberts, Mei-ling Wu, W. S. Hixon, and Elizabeth J. Crawford. 1954. The quantitative histochemistry of brain. II. Enzyme measurements. *J. Biol. Chem.* 207: 19–37.

Michelson, Ann, Elizabeth S. Russell, and P. J. Harman. 1955. Dystrophia muscularis: a hereditary primary myopathy in the house mouse. *Proc. Nat. Acad. Sc.* 41: 1079–1084.

Morton, N. E., and C. S. Chung. 1959. Formal genetics of muscular dystrophy. *Am. J. Human Genet.* 11: 360–379.

Morton, N. E. 1959. Genetic tests under incomplete ascertainment. *Am. J. Human Genet.* 11: 1–16.

Murphy, E. G., and M. M. Cherniak. 1958. Glutamic oxalacetic transaminase activity in the serum in muscular dystrophy and other neuromuscular disorders in childhood. *Pediatrics* 22: 1110–1113.

Pearson, C. M. 1957. Serum enzymes in muscular dystrophy and certain other muscular and neuromuscular diseases. 1. Serum glutamic oxalacetic transaminase. *N. England J. M.* 256: 1069–1075.

Schapira, G., M. Joly, and Fanny Schapira. 1953. L'élévation du taux de l'aldolase sérique: test biochemique des myopathies. *Sem. Hôp. Paris.* **29**: 1917–1920.

Schapira, Fanny, J.-C. Dreyfus, and G. Schapira. 1957a. Présence de deux aldolases de type différent dans le sérum. *C. rend Acad. sc.* **245**: 1–3.

Schapira, Fanny, J. Demos, G. Schapira, and J.-C. Dreyfus. 1957b. Facteurs de l'hyperaldolasémie au cours des myopathies. *Rev. Franc. Études Clin. Biol.* **2**: 728–734.

Schapira, G., J.-C. Dreyfus, F. Schapira, and J. Kruh. 1955. Glycogenolytic enzymes in human progressive muscular dystrophy. *Am. J. Phys. Med.* **34**: 313–319.

Sibley, J. A., and A. L. Lehninger. 1949. Determination of aldolase in animal tissues. *J. Biol. Chem.* **177**: 859–872.

Sibley, J. A., and G. A. Fleisher. 1954. The clinical significance of serum aldolase. *Proc. Mayo Clin.* **29**: 591–603.

Steinberg, D., D. Baldwin, and B. H. Ostrow. 1956. A clinical method for the assay of serum glutamic-oxalacetic transaminase. *J. Laborat. Clin. M.* **48**: 144–151.

Stevenson, A. C. 1953. Muscular dystrophy in Northern Ireland. I. An account of the condition in fifty-one families. *Ann. Eugen.* **18**: 50–93.

Swinyard, C. A., G. W. Deaver, and L. Greenspan. 1957. Gradients of functional ability of importance in rehabilitation of patients with progressive muscular and neuromuscular diseases. *Arch. Phys. Med. Rehab.* **38**: 574–579.

Van Meter, J. R. 1953. Progressive muscular dystrophy—A preliminary report on treatment with amino acids, folic acid and vitamins. *California M.* **79**: 297–299.

Walton, J. N. 1955. On the inheritance of muscular dystrophy. *Ann. Human Genet.* **20**: 1–38.

White, L. P. 1959. Serum enzymes. Variations of activity in disease of muscle. *California M.* **90**: 1–8.

Zierler, K. L. 1958. Aldolase leak from muscle of mice with hereditary muscular dystrophy. *Bull. Johns Hopkins Hosp.* **102**: 17–20.

Hosojima[1]

N. ISHIKUNI

Planning Section, Statistics Division, Hiroshima Prefectural Office

H. NEMOTO, J. V. NEEL,[2] A. L. DREW,[3] T. YANASE[4]

Child Health Survey

AND

Y. S. MATSUMOTO

Atomic Bomb Casualty Commission
Hiroshima, Japan

THE CIRCUMSTANCES which may lead to a relatively high rate of consanguinous marriage in a community are diverse. It is the purpose of the present note to describe briefly a small genetic isolate in which, due to unusual customs and traditions shortly to be described, the consanguinity rate appears to be the highest yet recorded for any group.

Hosojima is an irregularly shaped island, 1.64 kms. in length and .74 kms. in greatest width, on which in 1959 there lived 175 persons. Administratively it is a part of Innoshima City, Hiroshima Prefecture. To the north, approximately 2.0 kms., is the main Japanese island of Honshū, while 0.6 kms. to the south is the large island of Innoshima (population 41,164 in 1958), famous in local history as a former stronghold from which pirates sallied forth to plague the China Sea—and perhaps occasionally the more remote portions of feudal Japan itself. A steady stream of small coastal shipping passes the island on all sides. From the geographical standpoint the island is really less isolated than hundreds of similarly sized islands dotting the Inland Sea.

THE COEFFICIENT OF INBREEDING

The probability of a high rate of consanguineous marriage on the island became known to the senior author in the course of his responsibilities in the Planning Section of the Statistics Division of the Hiroshima Prefectural Office. Locally, the inhabitants of the island were known for their limited contacts with

[1] This investigation was supported in part by grants from the United States Atomic Energy Commission, the Rockefeller Foundation, and the Association for Aid to Crippled Children.

[2] Present address: Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan.

[3] Present address: Department of Neurology, University of Indiana Medical School, Indianapolis, Indiana.

[4] Present address: Department of Internal Medicine, Kyushu University Medical School, Fukuoka, Japan.

their neighbors and custom of marrying "within the island." Accordingly, field work was undertaken in January of 1959. There are 28 households on the island, within which there are to be found a total of 45 marriages with one or both marital partners surviving. Preliminary information concerning the frequency of consanguineous unions among these 45 marriages was obtained through an interview with seven individuals who comprised an "island council." This preliminary information was then carefully compared with the "koseki" (family) records for the island, as maintained at a branch office of the Innoshima City Hall. The value of this comparison is shown by the fact that whereas on the basis of history alone the rate of consanguineous marriage was estimated to be 19/45, this rate was definitely increased to 25/45 by the koseki check, and moreover many additional relationships for persons involved in marriages already known to be consanguineous came to light. In addition, the check of the koseki records raised questions concerning several possible additional consanguineous marriages. Accordingly, in May of 1959 the island was visited again, at which time four more consanguineous marriages were confirmed, for a total of 29/45, or 64.4 per cent. Five instances were encountered where a living individual had been married twice (families B, D, I, K, V); only the more recent marriage (conventionally placed to the right in Figure 1) was scored for consanguinity and included in the percentages given above.

The information concerning the occurrence and type of consanguinity in these marriages is summarized in Figure 1. Only the pedigrees in which a marriage involves inbreeding are shown. Because in 26 of the 28 households the head of the house bore the same surname, records were kept by mapping the location of each house on the island and identifying its occupants by a letter—this is the significance of the letters beside all but one of the pedigrees. A "total pedigree" for the present inhabitants of the village has been constructed but unfortunately does not lend itself to reproduction. It reveals a maze of interconnections which constitute an even more dramatic demonstration of the degree of inbreeding on the island than the isolated family pedigrees. It is noteworthy that although first-cousin marriages are the highest degree of consanguinity permitted by Japanese law, and hence, understandably, no closer marriages were encountered, in 11 of the 29 consanguineous marriages, because of multiple types of relationship, the coefficient of inbreeding among the offspring was greater than for a first-cousin marriage. Pedigrees X and Y contain the most extreme degrees of inbreeding encountered. In pedigree Y, for example, the coefficient of inbreeding for the offspring of the indicated marriage would be .10937, the individuals in the indicated marriage being related as first cousins, second cousins, second cousins once removed in three ways, and third cousins in two ways. The mean coefficient of inbreeding among the offspring of the 45 marriages represented on the island, assuming equal fertility of all marriages, would be .03341. This coefficient would appear to represent the highest yet recorded for a human community, although Shinozaki (1955) and Munakata (1955) have presented data on several Japanese villages which, while not suitable for the calculation of inbreeding coefficients, do indicate a degree of inbreeding approaching or even exceeding that observed on Hosojima.

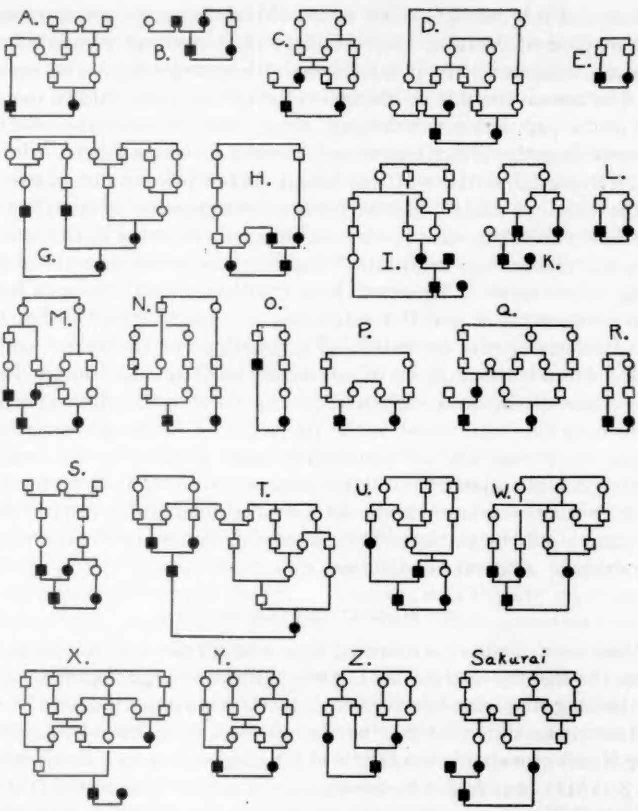

FIGURE 1. The types of consanguineous marriages encountered on Hosojima. Pedigrees are not given where no consanguinity could be established. The letters indicate house designation. The solid symbols indicate living individuals with whose marriages we are concerned. To conserve space, children of current marriages are not indicated.

In 17 houses there were representatives of two marriages, in each instance involving a parent-child (or foster child) relationship. In an effort to detect time trends, we have grouped marriages into "proximal" (P) and "distal" (P-1) generations, placing all marriages where only one is present in a household in the "proximal" group. The coefficient of inbreeding among the offspring of the 28 "proximal" marriages is .03808, and for 17 "distal," .02573. The lower frequency in the distal group would seem to indicate a trend towards an increasing frequency of consanguineous marriage on this island. Since, however, information is better for the "proximal" than the "distal" generation, it seems quite possible this apparent trend is spurious.

In most efforts to derive coefficients of inbreeding for populations, the coefficient has been based on an analysis of existing marriages with no reference to the number of children produced by these marriages. However, *sensu stricto*,

the coefficient of inbreeding applies to individuals, rather than marriages, and the usual method of deriving the coefficient of inbreeding yields an accurate figure for a population only if all classes of marriages have the same mean fertility. The reason for this short-cut is obvious: in most studies the fertility structure of the population is unknown. Since, however, we have an unusually complete genealogy for Hosojima, we can derive a mean coefficient of inbreeding for the 175 living inhabitants of this island. This coefficient is .02408, in contrast to the value of .03341 derived from a consideration of marriages alone. Taken at face value, this discrepancy would imply that the marriages characterized by the higher degrees of inbreeding are less fertile than those in which inbreeding is less marked. However, attention has already been called to the difference between the P and P-1 marriages as regards apparent degree of inbreeding. In deriving the coefficient of inbreeding for the entire population, one in effect extends the analysis of inbreeding back an addditional generation. An examination of the data suggests that the lower total population figure is a result of both this time trend in the frequency of consanguineous marriage, be it apparent or real, and an apparently lower fertility of the more inbred marriages, a complication of the latter impression being that in the proximal generation, with the greater apparent consanguinity rate, family size is in many instances still incomplete. The paucity of figures renders any more detailed analysis of no great significance.

THE HISTORY OF THE ISLAND

That these coefficients of inbreeding represent an absolute minimum is made certain by the history of the island. Although the desired degree of documentation is lacking, the islanders regard themselves as descendants of a samurai of the Matsumoto clan who, having the misfortune to have been allied with the losing Hosokawa clan in the Battle of Funaoka-yama in Yamashiro-no-kuni in Eishō 8 (1511), was forced to flee the site of action. It is stated that the first inhabitants of Hosojima, some 450 years ago, were this samurai (who had become a "mountain priest" in the tradition of vanquished samurai) and his bride, whom he acquired in the small settlement of Shigei on nearby Innoshima. The number of children born to this couple is unknown, but in the following generation the eldest son is said to have returned to his mother's birthplace, while at least one younger son remained behind on the island. The eldest son established the dominant branch of the family in Innoshima, while the younger son established a subsidiary branch on Hosojima. Family ties were maintained for many years through the feudal custom of the head of the subsidiary branch presenting annual gifts to the head of the dominant branch. Our history of Hosojima Island was obtained both from the islanders themselves and descendants of the dominant branch of the clan still residing on nearby Innoshima.

Some 300 years ago a second family, from the Hamamoto clan, came to the island. The head of this family is believed to have been a fugitive samurai from what is now Shimane Prefecture, but details are lacking. For the first several hundred years of its history, apparently only a handful of people lived

on Hosojima. It is alleged that because of the smallness of the island, there was a great reluctance of outsiders to settle there, in consequence of which there resulted over the years an extremely close degree of inbreeding, at times amounting to incest. Possibly the somewhat unsavory reputation Innoshima at one time enjoyed because of its reputation for piracy, referred to earlier, encouraged the inhabitants of Hosojima to avoid outside matrimonial alliances. That social exchange may be somewhat restricted in Innoshima itself (with consequences for Hosojima) is suggested by the relatively high rate of consanguineous marriage in parts of Innoshima (15 per cent in Ohama-mura; Sugimoto, 1950). In 1877, when in the course of the Meiji Restoration Japanese commoners acquired surnames, all of the inhabitants of Hosojima were given the surname Nishihara. In 1872, an additional family, later given the surname Sakurai, came to the island, while following World War II, a Nishihara woman who had married off the island and acquired the surname of Suzue returned with her husband. In 26 households the head now bears the surname Nishihara; in one, Suzue, and in one, Sakurai.

In feudal Japan the rights of primogeniture were rather strictly observed. This and other feudal institutions underwent rapid changes during the latter part of the nineteenth century. However, the Hosojima islanders did not follow the general trend. The island is devoted entirely to agriculture. Literally all of its farming activities are undertaken in cooperative form. To this day by tradition the eldest son inherits the farm—younger sons usually leave the island to find employment elsewhere. There are no hired laborers on the island. The eldest son finds his bride on the island if possible, a tradition apparently reinforced by the reluctance of "outside" women to share the isolation of the island. One tangible result of this policy has been that farm holdings on the island have not been fragmented to the degree encountered elsewhere in Japan; the average farm on Hosojima has an area about twice the present average for Hiroshima Prefecture. The island is relatively prosperous. Its inhabitants are not obliged to supplement their livelihood with part-time fishing, as is so often the case with island-dwelling families in Japan. A further unusual feature of the island's economy is that no rice is raised there. The principal crops are wheat, sweet potatoes, tobacco, and pyrethrum, the latter two, which occupy approximately 30 per cent of the acreage, being the "cash crops" that make possible the purchase of the necessary rice and fish.

From this brief sketch, it is apparent that the amount of remote inbreeding in the ancestry of this group must be high. The extent to which such remote inbreeding might contribute to the total coefficient cannot be estimated. The most nearly comparable study known to us is that of Spuhler and Kluckhohn (1953) on the Ramah Navaho Indians. This subdivision of the Navahos stems from a few families who moved into west central New Mexico about 1820 and who, aided to some extent by biological interchange with three other nearby Navaho communities, have now increased to over 600 individuals. Unusually favorable circumstances permitted the reconstruction of a total genealogy for the eight generations which this community has existed. It is significant from

the standpoint of the present inquiry that of the total coefficient of inbreeding for the population of .0066, some 10 per cent would have been unknown if the study had been restricted to degrees of relationship equal to or greater than third cousins. It can scarcely be doubted that the contribution of remote inbreeding to the true coefficient of inbreeding for Hosojima is at least of this magnitude and in all probability very significantly greater.

From the sociological standpoint, one of the outstanding evidences of the persistence on Hosojima of the old way of life is the survival of the institution of the "young people's group" (*seinendan*), which serves in time of fire or disaster, polices the beach, etc. The group has its own separate dormitory (*yado*) where the young bachelor males sleep every night. These youths carry on their individual work and eat their meals at their own farm household, and then gather at the dormitory every evening. The dormitory serves as a center for social recreation and a meeting place for young mixed company. Traditionally the group included only young men, but since 1936 all single males and females of the village, 16 to 25 years of age, are automatically members. At present the group includes 7 boys and 3 girls. The grammar school children gather in the early evening at the dormitory to be tutored by the young men and women. Within the recollection of the present inhabitants there has been no crime or suicide on Hosojima.

GENETICALLY DETERMINED DISEASE ON THE ISLAND

Circumstances did not permit a detailed evaluation of the physical and mental attributes of the islanders. However, at least two genetically determined conditions appear to have an unusual frequency on the island, as follows:

1. *Nerve deafness with muteness.*—Eight deaf or deaf-mute persons were examined. Three additional individuals said to be similarly affected were not seen. Thus, 6.3 per cent of the islanders exhibited some hereditary deafness. These individuals were found in eight sibships. For seven of the sibships, both parents were normal; in the eighth the mother, now deceased, was alleged to have been a deaf-mute. In the seven sibships where both parents were normal, the ratio of normal to affected among the living children was 13:10. The facts all point towards simple recessive inheritance of the condition and, in view of the antecedents of the islanders, a high probability that the same gene is involved in all cases, a fact of some interest in view of the variability in the clinical manifestations of the disease now to be described.

In the examination of the affected individuals, a set of matched tuning forks (frequencies of 128, 256, 512, 1024, and 2048) was used. Audiometric equipment was not available. Neurological examinations were performed on all, with particular attention to evidences of tempero-parietal lobe pathology as well as indications of congenital syphilis, feeblemindedness, or other evidences of diffuse brain damage. Otoscopic and ophthalmoscopic examinations were performed on all cases.

Three patients were not mute. In one of these bilateral otitis media with perforation of the left tympanic membrane was observed, a finding obviously

complicating the interpretation of this individual. In the second of these three cases a history suggestive of an otitis media at the age of 5 was obtained. However, our informants believed this child had some hearing loss prior to that time. The third patient who was not mute gave a history of the onset of hearing loss at age 16 with progression for two or three years and then no further change up to the present time (age 23). In all these cases examination revealed a partial bilateral nerve deafness. None had affected siblings.

One case of incomplete deaf-mutism was seen. Speech was limited; the 2048 tuning fork could be heard if the intensity was grossly increased. The remaining four cases were deaf-mutes whose hearing defect had been noted at an early age. They were found to have a nerve-type deafness characterized by the loss of hearing at all tuning fork frequencies or all but the lowest.

Three of the examined individuals were siblings, namely, the one incomplete deaf-mute and two of the four complete deaf-mutes referred to in the preceding paragraph. The individual referred to above with loss of hearing between the ages of 16 and 19 and then no further progression is a first cousin once removed of these three individuals. There is thus marked variability in the expression of this gene even in a population which from the genetic standpoint should be one of the most uniform ever described. Lindenov (1945) in his extensive study of deaf-mutism has emphasized the variability in the manifestations of the responsible gene. None of the affected persons showed significant associated disease.

2. *Dyschromatosis symmetrica hereditaria.*—Three individuals were examined with a rare hereditary pigmentary anomaly which was first described in Japanese in 1924 independently by Komaya and Matsumoto and given the name by which it is now known by Tōyama in 1929. So far no cases have been observed outside of Japan. The anomaly appears to be a complex of slightly increased pigmentation and depigmentation. It is not present at birth but usually develops during the first decade, characteristically appearing first on the dorsum of the hands and feet, then involving the distal portions of the extremities except for the palms and soles. In exceptional cases virtually the entire body may be involved. The pigmented areas within the depigmented regions vary widely in size and shape; in our three cases the majority ranged between 3 and 5 mm. in diameter. Sometimes the involved areas present a reticular appearance. The individuals whom we observed were characteristic of the anomaly as described by the above quoted authors and also by Ichikawa and Hiraga (1933), Ikuta (1940), Hoshimoto (1944), Itō (1950), Kano (1956) and Katō (1957). There are a total of 11 individuals with the condition on the island.

Two of the affected individuals examined were related as father and daughter. One of the five siblings of the latter was reported to be affected. The father's father and paternal grandmother, both deceased, were reported affected. The third affected person examined has five children, two reported affected. He is related to the first two individuals as second cousin and second cousin once removed, respectively. Although the disease was not known to be present in the persons through whom this relationship occurs except as stated above, the

information on this point is inadequate. These data would suggest a dominant mode of inheritance. However, several of the other affected individuals on the island are alleged to have normal parents. Unfortunately, we were unable to examine these parents ourselves, but if the report is correct, the possibility of the pseudodominant type of inheritance seen at high frequencies of a recessive gene should be considered. Aside from slight cosmetic considerations, the condition imposed no handicap on affected individuals.

Single cases of feeblemindedness with epilepsy in a child, Parkinsonism in an elderly female, and coloboma iridis in a middle-aged female were encountered. In no instance was there a positive family history. Circumstances did not permit a thorough survey for genetically-determined disease on the island, nor would such a survey have been helpful in the assessment of children dying at an early age which is so important to any attempt to evaluate the picture of genetically determined pathology in a population. The superficial impression of the children and adults seen on the island was of a range of variation no greater than commonly encountered in any small community, but the shortcomings of such superficial impressions are too well known to require any comment.

DISCUSSION

It would be unwise to generalize from Hosojima to patterns of inbreeding in Japan in past centuries. Even in feudal Japan the degree of inbreeding on this island was probably exceptional. On the other hand, to the extent that the island does represent a survival of certain feudal customs, it provides some insight into the degree of inbreeding in the rural areas and small islands of Japan only some four generations ago. It should be emphasized that despite the somewhat isolated location of the island, the factors responsible for this exceptional rate of inbreeding are social rather than geographical.

In view of the rapid decline of consanguineous marriage in contemporary Japan (Schull, 1958), it is apparent that a very significant relaxation of inbreeding has occurred in Japan in the past century, and that while genetic formulae which imply equilibrium are still useful in obtaining first approximations to gene frequencies, the limitations on their use should be constantly borne in mind.

SUMMARY

The population of a small island located in the Inland Sea of Japan has been described. On the basis of the analysis of all marriages in which one or both marital partners are alive, the coefficient of inbreeding in this population is .03341. Two hereditary diseases, nerve deafness with muteness and dyschromatosis symmetrica hereditaria, have a noteworthy frequency.

REFERENCES

HOSHIMOTO, T. 1941. Dyschromatosis symmetrica hereditaria. (Japanese) *Nippon Hifu-Hinyōki-Gaku Zasshi* (*Jap. J. Derm. Urol.*) 49: 316–318.

ICHIKAWA, T., AND HIRAGA, Y. 1933. Dyschromatosis universalis hereditaria. (Japanese) *Nippon Hifu-Hinyōki-Gaku Zasshi* (*Jap. J. Derm. Urol.*) **34: 360–364.**

IKUTA, T. 1940. Dyschromatosis symmetrica hereditaria. (Japanese) *Nippon Hifu-Hinyōki-Gaku Zasshi* (*Jap. J. Derm. Urol.*) **48: 82.**

ITŌ, M. 1950. Genetical Studies on Skin Diseases. I. Ephelides, Dyschromatosis symmetrica hereditaria and Xeroderma pigmentosum. *Tōhoku J. Exp. M.* **53: 69–72.**

KANO, E. 1956. Histological observations on dyschromatosis symmetrica hereditaria and allied diseases. (Japanese) *Hifubyō Baidoku-Gaku Zasshi* (*Jap. J. Derm. Syphil.*) **66: 392.**

KATŌ, T. 1957. Dyschromatosis symmetrica hereditaria. (Japanese) *Hifuka Kiyō* (*Derm. Bull.*) **52: 50.**

KOMAYA, G. 1924. Symmetrische Pigmentanomalie der Extremitaten. *Arch. Derm. Syph.* **147: 389–393.**

LINDENOV, H. 1945. The etiology of deaf-mutism with special reference to heredity. *Opera ex Domo Biolgiae Hereditariae Humanae Universitatis Hafniensis.* **8: 268** pp. Copenhagen: E. Munksgaard.

MATSUMOTO, S. 1924. Leukopathia punctata et reticularis symmetrica. (Japanese) *Hifuka Kiyō* (*Derm. Bull.*) **2: 191–197.**

MUNAKATA, T. 1955. Studies on consanguineous marriage in a mountainous district in Fukushima Prefecture, Japan. (Japanese) *Fukushima Igaku Zasshi* (*Fukushima Acta Medica, Japan*) **5: 131–136.**

SCHULL, W. J. 1958. Empirical risks in consanguineous marriages: Sex ratio, malformation, and viability. *Am. J. Human Genet.* **10: 294–343.**

SHINOZAKI, N. 1955. Eugenic research on a village of consanguineous marriage—Narata, Nishyama-Mura, Yamanashi Prefecture. *Jinkō Mondai Kenkyūsho, Shiriyō* (*Material Report of Institute of Population Problem*) **102: 1–24.**

SPUHLER, J. N., AND KLUCKHOHN, C. 1953. Inbreeding coefficients of the Ramah Navaho population. *Human Biol.* **25: 295–317.**

SUGIMOTO, T. 1950. The frequency of consanguineous marriage in a pure farm village on an island in the Inland Sea. (Japanese) *Igaku To Seibutsugaku* (*Medicine and Biology*). **16: 49–51.**

TŌYAMA, I. 1929. Dyschromatosis symmetrica hereditaria. (Japanese) *Nippon Hifu-Hinyōkika Zasshi* (*Jap. J. Derm. Urol.*) **29: 95–96.**

Studies of Alcaptonuria: Inheritance of 47 Cases in Eight Highly Inter-Related Dominican Kindreds*

ROBERT AUSTIN MILCH

From the Division of Orthopaedic Surgery, Department of Surgery, The Johns Hopkins University School of Medicine and the Johns Hopkins Hospital, Baltimore, Maryland

ALCAPTONURIA, the first of the genetically determined "inborn errors of metabolism" (6, 7, 11) to be described in man, is defined by the abnormal urinary excretion of an otherwise normal intermediary metabolite, homogentisic (2,5-dihydroxyphenylacetic) acid. Homogentisic aciduria arises in consequence of the absence of the enzyme homogentisic acid oxidase, and is almost always present at birth. In virtually all reported cases affected individuals later develop ochronosis (28), a clinical condition characterized by pathological pigmentation and degenerative phenomena in the connective tissues, primarily of the integumentary, ocular, cardiovascular and skeletal systems (3, 4, 12, 15, 20, 21, 26, 29). Subsequently, a highly significant percentage of patients develop more or less typical degenerative joint disease, or osteoarthritis, in widely distributed joints, particularly those of the axial and weight bearing portions of the skeleton, (1, 2, 8, 23) and various degenerative alterations in the eye and in the cardiovascular system (10, 27). The pathologic picture of all components of the alcaptonuria syndrome is that of a generalized metabolic disorder which diffusely, but secondarily affects the mesodermal connective tissues. As such, it tends to resemble somewhat certain other inherited primary disorders of the connective tissues, (13) such as gargoylism (Hunter-Hurler syndrome), pseudoxanthoma elasticum, osteogenesis imperfecta and the Ehlers-Danlos and Marfan syndromes. In contradistinction to these latter three entities, however, the alcaptonuria syndrome appears to be inherited not as a dominant, but rather as a simple, autosomal recessive.

A number of reported families, (14, 18, 24, 25), however, appear to contradict this view. Accordingly, alternate interpretative possibilities were suggested; namely that the alcaptonuria allele (a) may behave as a dominant "in a restricted range of variation", (9) or (b) may be so modified as to co-exist with

* Aided by a grant (A-2642) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Department of Health, Education and Welfare.

Presented, in part, at the annual meeting of the American Society of Human Genetics, State College, Pa., September 1, 1959.

an additional pair of gene factors to produce an apparent incompletely penetrant dominant gene substitution (16).

The fact that the family reported by Pieter (22), which is generally considered to be the sole apparently unequivocal instance of dominant inheritance in the literature, derived from the same geographical area as the family studied by Milch and Milch (15, 17), and subsequently reported again by Galdston et al., (5)* suggested the obvious possibility that the two families might be inter-related. Accordingly, an investigation of all known members of the two pedigrees was conducted in an attempt to provide a means whereby an adequate evaluation of the various genetic postulates could be made.

MATERIAL AND METHODS

Information concerning family relationships was obtained from members of the families living in various cities, towns, and villages in the Dominican Republic. This was repeatedly re-checked both with other members within the family and from numerous public and private records. Surviving members of the families were questioned concerning the clinical stigmata of simple alcaptonuria, ochronosis, alcaptonuric arthritis and arteriosclerosis, and were examined both clinically and chemically for the presence of any feature of the alcaptonuria syndrome. All individuals stated to have been alcaptonuric and so noted in the accompanying pedigree charts have had urine and clinical examinations with but three exceptions. Two of these individuals (III-6 and IV-12) were deceased at the time of the study, but circumstantial evidence compellingly indicated that they were alcaptonuric. The third (VI-16) refused examination, but on inspection was obviously ochronotic.

The clinical history was accepted as positive if the subject or any relative noted (a) darkening of the urine on exposure to the air, brownish staining of diapers or undergarments and brown or brown-green discoloration of the skin of the axillae, etc., on perspiration (*simple alcaptonuria*) or (b) bluish discoloration of the sclerae, tendons or nasal or aural cartilages (*ochronosis*).

A positive laboratory diagnosis of alcaptonuria was made when the urine turned dark on standing in air or upon addition of alkali (0.1 N NaOH), reduced copper solutions (Clinitest (R), Fehling's and Benedict's) and gave a positive Pieter-Fishberg test with x-ray film.

Once the identity of alcaptonuric individuals had been ascertained, their precise family relationship was again checked with various members of the families and the inter-relationships of parents and siblings established with certainty. The relationship of each individual to the eight distinct kindreds of

---

* One of the patients reported by Galdston et al. (5) had previously been noted by both Smith (27) and Pomeranz et al. (23); another had been noted previously by Milch and Milch (15). Follow-up data on the families reported by Osler (21) and both Smith (27) and Pomeranz et al. (23) were later re-evaluated by Milch (18).

Anatomical findings in the patients reported by Coodley and Greco (3) were later described in detail by Lichtenstein and Kaplan (12).

the study was analyzed with respect to the basic family inter-relationship and the pedigree chart was then constructed.

RESULTS AND COMMENTS

Initial efforts were directed toward establishing the inter-relationship of the family reported by Pieter (22) with that later reported (15). Concerted collaborative efforts on the parts of Dr. Pieter, Dr. Henry Milch and this writer over a period of years were, however, not entirely successful. Some members of the 1925 pedigree were identified, but by far the greatest number could not be identified at all. Thus, though the present pedigree unquestionably includes a number of unaffected members of the families reported by Pieter in 1925, it does not include all members, affected or unaffected, reported at that time. So far as could be ascertained only two alcaptonuric individuals (III-6 and IV-12, both of whom were deceased at the time of this study) noted in the present report were possibly, though not certainly, included in the 1925 genealogy.

Eight different surnames were encountered among the inter-related families of the present study. These are identified by encircled arabic numerals in Figure 1. Precisely known relationships have been indicated by solid lines. Known and unequivocal relationships, but in which the exact mechanism of the relationship remains uncertain, have been indicated by broken lines. Of these eight kindreds, seven could be accurately traced. The origin of this eighth kindred, could not be traced beyond the V generation, but it is clearly related to kindreds 6 and 7 (Figure 1) (*see addendum*).

As far as homogentisic aciduria is concerned nothing is known about the status of any of the individuals of generations I, II and III with the exception of III-6. This individual is known to have been clinically alcaptonuric and to have suffered from both ochronosis and alcaptonuric arthritis. All of the individuals in the subsequent generations have been examined.

The original parent (I-1) noted in the pedigree had five known matings, of which only two have been diagrammed here. No information could be obtained from any source concerning the other three matings, nor of any of the descendants of these particular matings, despite the fact that the eight kindreds in question all live or have lived within a relatively small geographical area in the Dominican Republic. Consequently, it is possible that there are even more inter-relationships between the kindreds than have been noted in the pedigree (Figure 1).

The union of III-1 and II-3 resulted in the birth of seven children, of whom IV-1 is the original parent (II-1) in the initially reported pedigree (15). This individual, deceased at the time of the initial report, was believed to have been alcaptonuric and was so reported (15). During the course of the present investigation however, a great deal of circumstantial evidence was obtained which tends to suggest that this individual was, on the contrary, normal, though no data concerning his biochemical status is available (19). He married a non-affected, non-relative, IV-2, and had eight children. Three of these, V-3 and V-6, died during neonatal life and there is no information concerning their

status so far as alcaptonuria is concerned. V-4, also initially considered to be alcaptonuric, has been examined and has been found to be normal (19). Thus, of the eight children of IV-1 and IV-2, only two (V-2 and V-5) are definitely established as having the metabolic disorder.

V-5 (indicated by the arrow) represents the proband and represents subject III-5 of the 1951 report (15). He is known to have had two non-consanguineous matings. One of these resulted in a normal female; the other produced a normal male, who married a non-relative and had four normal children. V-2 married a non-relative and had seven children and sixteen grandchildren (VII-2, VII-3) all of whom are normal.

V-7, a brother of the proband, married his first cousin, V-8. Of their four children, two, VI-4 and VI-5, are alcaptonuric. VI-5 married a non-relative and had two daughters, both of whom are normal. VI-4 is not married. The descendants of the remaining siblings of IV-2 and IV-3 are all normal, even though there has been a consanguineous marriage in each of generations IV and V.

V-12 and V-13, two males among seven siblings born to the union of IV-7 and IV-8, are affected. There are no subsequent descendants of this particular mating, though a second mating of IV-8 is known to have resulted in one female child, thirteen grandchildren and nine great-grandchildren, all of whom are normal. A great-great-grandchild however, VIII-1, is alcaptonuric. There is some question as to the possible family inter-relationships existing between the mother of VIII-1 and other members of the study kindreds, though some members of the family maintain that this individual is a non-relative.

Although the precise relationship between IV-7 and V-14 is not clearly established, it is established that the two individuals are directly related. V-14 also had an alcaptonuric son (VI-7) as the result of a mating with a woman whose sister likewise had an alcaptonuric son and whose grandmother (III-6) was similarly affected.

The inter-relationships of the remaining affected individuals in the pedigree presented here (Figure 1 and Table 1) are concerned primarily with the kindreds 4, 5, 6, 7 and 8. Although inextricably related to them, these latter kindreds are more or less distinct from the kindreds 1, 2 and 3 and differ from them in two prime considerations. Firstly, the kindreds 1, 2 and 3 either migrated to urban communities or initially settled the larger cities of the country; secondly, as regards matings, they tended to maintain the original Castillian origin of their respective families in more or less undiluted concentration. On the other hand, kindreds 4, 5, 6, 7 and 8, and especially the apparently distinct eighth kindred, have tended to remain in the relatively isolated rural communities and early to have participated in mixed matings between the three ethnic groups (Caribe Indian, Negro and Caucasian) originally present on the island.

Extra-marital matings and illegitimacies were quite common in each of the eight kindreds and, where pertinent and known, have been indicated in the pedigree (Figure 1). In general, these occurred more frequently in kindreds 4,

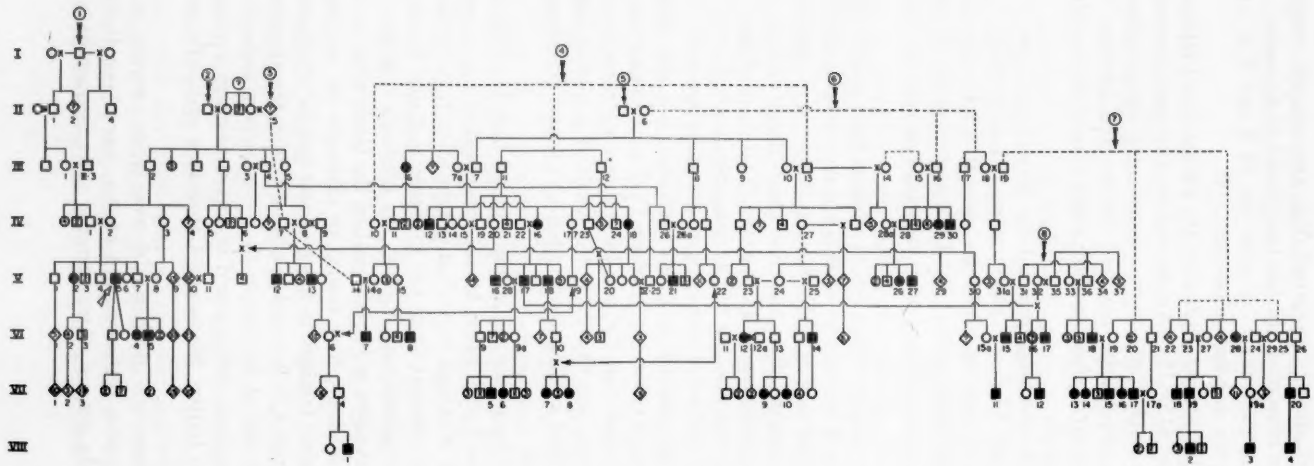

FIG. 1. Pedigree of the eight inter-related kindreds. None of the individuals in generations I, II and III has been examined; all of the remaining individuals, however, have been examined. Standard symbols are employed except that matings are indicated in places by crosses between the two involved individuals.

TABLE 1. SUMMARY OF KNOWN MATINGS BETWEEN APPARENT NORMALS PRODUCING ALCAPTONURIC OFFSPRING

Mating A × B		Number of Offspring	Number Alcaptonuric	Identity of Alcaptonuric Offspring	Fraction Alcaptonuric
III-7	III-7a	7	3	IV-12,16,18	0.43
III-15	III-16	11	2	IV-29,30	0.18
IV-1	IV-2	8	2	V-2,5	0.25
IV-7	IV-8	7	2	V-12,13	0.29
IV-26	IV-26a	5	1	V-21	0.20
IV-28	IV-28a	8	2	V-26,27	0.25
V-7	V-8	4	2	VI-4,5	0.50
V-14	V-14a	1	1	VI-7	1.00
V-15	—	6	1	VI-8	0.17
V-24	V-23	2	1	VI-12	0.50
V-24	V-25	2	1	VI-14	0.50
V-31	V-31a	5	1	VI-15	0.20
V-33	V-36	7	1	VI-18	0.14
VI-9	—	6	1	VII-5	0.17
VI-9a	—	6	1	VII-6	0.17
VI-10	V-22	5	2	VII-7,8	0.40
VI-13	—	3	2	VII-9,10	0.67
VI-23	VI-27	8	2	VII-18,19	0.25
VI-26	—	2	1	VII-20	0.50
VII-4	—	2	1	VIII-1	0.50
VII-19a	—	1	1	VIII-3	1.00

5, 6, 7 and 8, though were also noted in kindreds 1, 2 and 3. For socio-economic reasons which are not germane to the present discussion, the extra-marital matings of the males of kindreds 1, 2 and 3 tended to occur with the females of kindreds 4, 5, 6, 7 and 8.* Correspondingly, the incidence of alcaptonuria was greater in kindreds 4, 5, 6, 7 and 8 than in kindreds 1, 2 and 3. As a corollary of the clearly greater alcaptonuric gene pool in kindreds 4, 5, 6 and 7, all six of the instances of direct transmission of the disorder from parent to child have been observed in these kindreds (Tables 1 and 2).

IV-16, one of three alcaptonuric siblings (IV-12, IV-16 and IV-18), married a distant relative (IV-22) and three of her nine children (V-16, V-17, and V-18) are alcaptonuric. Two of these individuals (V-17 and V-18) are grandparents of alcaptonurics. In addition, V-17 also sired two alcaptonuric children (VI-16 and VI-17), one of whom has in turn had an alcaptonuric son (VII-12).

VI-15, a first cousin of VI-16 and VI-17, likewise has had an alcaptonuric child (VII-11), VI-18, also a first cousin of VI-16 and VI-17 sired five alcap-

* Presumably other extra-marital matings have also occurred which have not been elucidated, and hence a mathematical evaluation of the frequency of appearance of the homozygous state (Figs. 2 and 3) may add little and in fact be misleading. In order to provide additional data on the details of some of the questionable family relationships, a biochemical study of serum and red cell proteins among these individuals is currently in progress.

TABLE 2. SUMMARY OF KNOWN MATINGS OF ALCAPTONURIC INDIVIDUALS

Mating		Number of Offspring	Number Alcaptonuric	Identity of Alcaptonuric Offspring	Fraction Affected
Alcap. × Normal					
III-6	—	5	0		0.0
IV-16	IV-22	9	3	V-16,17,18	0.333
IV-18	—	7	0		0.0
V-2	—	7	0		0.0
V-5	—	1	0		0.0
V-5	—	2	0		0.0
V-17	V-28	11	0		0.0
V-17	V-32	2	2	VI-16,17	1.0
V-18	—	8	0		0.0
VI-5	—	2	0		0.0
VI-12	VI-11	3	0		0.0
VI-12	VI-12a	2	0		0.0
VI-14	—	1	0		0.0
VI-15	VI-15a	1	1	VII-11	1.00
VI-16	—	2	1	VII-12	0.50
VI-18	VI-19	8	5	VI-13,14,15, 16,17	0.625
VI-28	VI-24	12	0		0.0
VII-17	VII-17a	4	0		0.0
VII-19	—	7	2	VIII-2	0.186
VII-20	—	1	1	VIII-4	1.00

tonuric children (VII-13, VII-14, VII-15, VII-16 and VII-17). As regards this inter-relationship, it is significant to note that the father (V-31) of VI-15 is a brother both of V-32, the mother of VI-16, and V-33, the mother of VI-18.

The remaining instance of direct transmission from parent to offspring involves the alcaptonuric son (VIII-4) of an alcaptonuric father (VII-20), who is alleged to have married a non-relative, though this is not at all certain.

Thus, two of the six instances of direct transmission have occurred in three successive generations of the descendants of one sibship and four have occurred in consequence of matings with members of one specific sibship. The remaining instance, apparently not directly related to either of these, is almost certainly indirectly related.

Kindreds 1, 2 and 3, comprising affected individuals V-2, V-5, V-12 and V-13 and VI-4 and VI-5, pose no special interpretative difficulties. The allele is clearly not manifested as a simple dominant and the observed ratios of affected to unaffected individuals closely correspond to what would be expected if in fact the allele were manifested as a simple recessive (Figure 1, and Tables 1 and 2).

Kindreds 4, 5, 6, 7 and 8 and that related to both 6 and 7, present a somewhat more complex situation. These too, however, can be readily accounted for on the basis of the simple, recessive hypothesis. In those instances in which neither parent was affected, the observed data closely approximate that to be expected if it be assumed that both parents were heterozygous for the abnormal allele.

Similarly, where one parent of affected children is also affected, the observed frequency ratios are of an order of magnitude closely corresponding to those to be expected if it be assumed that the union involved an individual heterozygous for the abnormal allele and one homozygous for the allele (Figure 1, and Tables 1 and 2).

In terms of the observed degree of consanguinity, it is entirely to be expected that the higher ratios of alcaptonuric subjects to normal individuals tend to have occurred in kindreds 4, 5, 6, 7 and 8. Furthermore, all of the cases of direct transmission from parent to offspring, which produce apparent, though spurious dominant inheritance occur in precisely these same kindreds.

Despite a significantly high incidence of cerebral dysfunction or frank mental retardation and of umbilical, inguinal and femoral herniae in multiple instances of several segments of the study kindreds, the present data nevertheless appear to be explicable on the basis of a simple allele hypothesis. Viewed collectively, therefore, all of the observed data appear to be accounted for by the assumption that the alcaptonuria allele behaves in this, as in other reported pedigrees, as if it were a simple, rare, autosomal recessive.

SUMMARY

A study has been conducted of eight highly inter-related kindreds segregating for alcaptonuria in the Dominican Republic. Forty-seven patients have been identified in eight generations of these kindreds. The observed data appear consistent with the hypothesis that the allele is manifested as a simple, rare, autosomal, recessive, despite apparent dominant transmission in several successive generations in one highly inter-related segment of the presented pedigree.

ADDENDUM

Since this paper was submitted for publication it has been possible to re-examine the study kindreds and to corroborate the presently reported data. Furthermore, it has been ascertained that the eighth kindred, referred to herein as "apparently distinct . . . (but) . . . clearly related to kindreds 6 and 7," is in point of fact related to kindreds 6 and 7 on both the maternal and paternal sides. Thus, there are 47 cases in but *seven* highly inter-related kindreds. Data on this relationship, including a report of 11 additional cases, will be presented separately.

ACKNOWLEDGMENT

A great many people have cooperated with the writer in making the present study not only possible but also informative and exact. Many of these are unnamed, but to them, just as earnestly as to those noted here, he wishes to express his sincere thanks. In particular, he wishes to acknowledge the active collaboration, over a period in excess of eight years, of Dr. Henry Milch of New York and of Dr. Heriberto Pieter of Ciudad Trujillo, Dominican Republic, without whose efforts the study would never have been conducted no less completed. Also, he wishes to acknowledge his indebtedness to Doctors Robert A. Robinson, Victor A. McKusick and Samuel H. Boyer IV, of the Johns Hopkins University

and Hospital for their helpful suggestions and aid regarding the manuscript; to Messrs. Pericles A. Franco and Raphael Perello and to Doctors Amiro Perez, Deloros A. de la Huerga and Alphonso Simpson, of Ciudad Trujillo, R.D. and Dr. Lorenzo Pellerano, Santiago, R.D. for their aid during the course of the study, and to Miss Susan Brown for her secretarial assistance.

REFERENCES

(1) Albrecht, H. 1902. Ueber ochronose. *Zschr. Heilk.* (path. Anat.) 23: 366–378.

(2) Allard, E., and Gross, O. 1909. Alkaptonurie und ochronose. *Mitt. Grenzgeb. Med. Chir.* 19: 24–32.

(3) Coodley, E. L., and Greco, A. T. 1950. Clinical aspects of ochronosis with report of a case. *Am. J. Med.* 8: 816–822.

(4) Cooper, J. A., and Moran, T. J. 1957. Studies on ochronosis. I. Report of case with death from ochronotic nephrosis. *Arch. Path.* 64: 46–53.

(5) Galdston, M., Steele, J. M., and Dobriner, K. 1952. Alcaptonuria and ochronosis with a report of three patients and metabolic studies in two. *Am. J. Med.* 13: 432–452.

(6) Garrod, A. E. 1902. The incidence of alkaptonuria: A study in chemical individuality. *Lancet* 1: 1616–1620.

(7) Garrod, A. E. 1923. *Inborn Errors of Metabolism*. Second Edition, London: H. Frowde, Hodder and Stoughton.

(8) Hertzberg, J. 1945. On osteoarthritis alkaptonurica (ochronotica) with description of one case. *Acta radiol.* 26: 484–490.

(9) Hogben, L., Worrall, R. L., and Zieve, I. 1932. The genetic basis of alkaptonuria. *Proc. R. Soc. Edinburgh* 52: 264–295.

(10) Khachadurian, A., and Abu Feisal, K. 1958. Alkaptonuria. Report of a family with seven cases appearing in four successive generations, with metabolic studies in one patient. *J. Chron. Dis.* 7: 455–465.

(11) Knox, W. E. 1958. Sir Archibald Garrod's "Inborn Errors of Metabolism" II. Alkaptonuria. *Am. J. Human Genet.* 10: 95–124.

(12) Lichtenstein, L., and Kaplan, L. 1954. Hereditary ochronosis. Pathological changes observed in two necropsied cases. *Am. J. Path.* 30: 99–126.

(13) McKusick, V. A. 1956. *Heritable Disorders of Connective Tissue*. St. Louis: C. V. Mosby Co.

(14) Martin, W. J., Underdahl, L. O., Mathieson, D. R., and Pugh, D. G. 1955. Alkaptonuria: Report of 12 Cases. *Ann. Int. M.* 42: 1052–1064.

(15) Milch, H., and Milch, R. A. 1951. Alcaptonuria. *J. Internat. Coll. Surgeons* 15: 669–685.

(16) Milch, R. A. 1955. Direct inheritance of alcaptonuria. *Metabolism* 4: 513–518.

(17) Milch, R. A., and Milch, H. 1957. Dominant inheritance of alcaptonuria. *Acta Genet.* 7: 178–184.

(18) Milch, R. A. 1958. Inheritance of alcaptonuria. *Bull. Hosp. Joint Dis.* 18: 103–111.

(19) Milch, R. A. 1959. A preliminary note of 47 cases of alcaptonuria occurring in seven inter-related Dominican families, with an additional comment on two previously reported pedigrees. *Acta Genet. et Stat. Med.* 10: 123–126.

(20) Oppenheimer, B. S., and Kline, B. S. 1922. Ochronosis, with a study of an additional case. *Arch Int. M.* 29: 732–747.

(21) Osler, W. 1904. Ochronosis: The pigmentation of cartilages, sclerotics and skin in alkaptonuria. *Lancet* 1: 10–11.

(22) Pieter, H. 1925. Une famille d'alcaptonuriques. *Presse méd.* 33: 1310.

(23) Pomeranz, M. M., Friedman, L. J., and Tunick, I. S. 1941. Roentgen findings in alkaptonuric ochronosis. *Radiology* 37: 295–303.

(24) Sitaj, S., and Urbanek, T. 1956. Alkaptonuria. *Rev Czech. Med.* 2: 288–292.

(25) Sitaj, S., Cervenansky, J., and Urbanek, T. 1956. Alkaptonuria a Ochronoza Vydavatel'stvo Slovenské j Akademie Vied., Bratislava.

(26) Skinses, O. K. 1948. Generalized ochronosis. Report of an instance in which it was misdiagnosed as melanosarcoma, with resultant enucleation of an eye. *Arch. Path.* **45**: 552–558.

(27) Smith, J. W. 1942. Ochronosis of the sclera and cornea complicating alkaptonuria. *J. Am. M. Ass.* **120**: 1282–1288.

(28) Virchow, R. 1866. Ein fall von allgemeiner ochronose der knorpel und knorpelaehnlichen theile. *Virchows Arch.* **37**: 212–219.

(29) Young, H. H. 1944. Calculi of the prostate associated with ochronosis and alkaptonuria. *J. Urol.* **51**: 48–58.

Blood Groups of Caddoan Indians of Oklahoma

MARGERY P. GRAY AND WILLIAM S. LAUGHLIN

Department of Anthropology, University of Wisconsin, Madison, Wisconsin

INTRODUCTION

TRIBES CLASSIFIED as belonging to the Caddoan linguistic family present problems in tracing their prehistoric movements and locations. In the latter part of the 19th Century the Pawnee were moved from central Nebraska to northern Oklahoma and the Caddo were moved from Texas and Louisiana up to central Oklahoma to an area claimed at that time by the Wichita. At present, the Pawnee are concentrated in the vicinity of Pawnee, Oklahoma. The Wichita and Caddo are located in the vicinity of Anadarko, Gracemont and Binger, Oklahoma. All three tribes have undergone drastic population reductions since white contact. Population estimates based on Mooney's 1780 figures list 10,000 Pawnee and 13,400 Red River Caddoans (Caddo, Wichita, Kichai and Waco) (Kroeber, 1947). At the time of contact, the Pawnee were composed of 4 endogamous bands: Skidi, Kitkahaxhi, Pitahauriata, and Chaui. The largest, Skidi, maintained 13 villages (Lowie, 1954). The old band endogamy broke down with the enforced move from Nebraska. Each band still maintains a cemetery in the vicinity of Pawnee even though the biological significance of band membership has disappeared. The present tribal roles list about 1500 Pawnee but many of these are less than ¼ Pawnee. A total of 160 Pawnee were blood typed.

The Caddo are the remnants of a loose federation of groups whose territory extended from the west bank of the Mississippi River in Louisiana and along the Red River in Texas (Kroeber, 1947). It is not known how far north the Caddo extended. At the present time the Caddo number not more than 250 with many of those being hybrids. Of the 250, 61 Caddo were blood typed.

At the time of contact, the Wichita were apparently occupying an area which brought them in contact with the Caddo to the south. The northern limit is undefined except that it is known that they extended up into Kansas (Kroeber, 1947). The latest census lists 515 individuals of which 235 are listed as 4/4 Wichita, 122 as 3/4 Wichita, 76 as 1/2 Wichita, 68 as 1/4 Wichita, and 14 as not more than 1/8 Wichita. A total of 137 Wichita were blood typed.

At the present time the Pawnee and Wichita maintain close cultural ties in the form of reciprocal hand games, dances, and gift exchanges. No such ties were detected between the Pawnee and Caddo. The cultural affinities of the Caddo and Wichita may be a reflection of having lived in the same area since moved by the government or may have extended back into prehistory.

This project was supported by the Wenner Gren Foundation and the Graduate School of the University of Wisconsin.

Received July 13, 1959.

DESCRIPTION OF SAMPLE

Human groups form Mendelian populations if they set themselves apart from surrounding groups and maintain their distinctiveness by language or social customs which encourage marrying within the group. Boundaries of such populations will be sharp or diffuse depending on the strength of social pressures. If the size of an intra-breeding population is small, many of its members will be related to one another. Collecting genealogical information on nuclear families will prevent sibs, and parents and offspring from being included in a sample; however, extensive biological relationships of extended families will be missed and mistakenly be presumed to be absent. The extent of intrarelationship in a small group makes it impossible to obtain an adequate sample of unrelated individuals. Since random mating is not possible in a small group, it is impossible to select a random sample. Sub-sample is the term that will be used in this paper. Since the degree of relationship of individuals included in the sample has a marked effect on phenotype frequency (Table 1), the coefficient of relationship between all individuals was determined. The greatest coefficient of relationship (r) allowed in the computation of gene frequencies of Caddo, Wichita, and Pawnee was $r = .125$. The coefficient of relationship was computed by the formula $r = 1/2^{n}$ where n = the number of biological links connecting any two individuals. As an example, the value of r between parents and offspring would be .500. A smaller value of r would have reduced the sample size to the point where statistical calculations would have been impossible for any of the three groups.

Since gene frequencies estimated from the present sub-samples were to be compared with gene frequencies of other groups, attention was paid to the extent that tribal membership followed biological membership. There is an increasing tendency for Indian tribes to adopt entire families of non-Indians. In the present instances, individuals of Mexican ancestry could not have been excluded on purely morphological grounds.

"Full bloods" in the present samples reported all four grandparents with the same tribal affiliation. Tribal samples are composed of "full bloods" plus Pawnee, Wichita or Caddo—Indian hybrids. All Indian-non-Indian hybrids were excluded since their biological backgrounds were not as clear.

The Pawnee tribal sample is composed of "full bloods" plus Wichita, Ponca, Kiowa, Oto, Sauk and Fox, Cree, Cherokee, Potawotomi, Osage, Blackfoot, Quapaw, Iowa, Arapaho, Wyandotte and Chinook hybrids. The Wichita tribal sample is composed of "full bloods" plus Caddo, Pawnee, Kiowa, Commanche, Seneca, Creek and Delaware hybrids. The Caddo tribal sample is composed of "full bloods" plus Wichita, Commanche, Delaware, Choctaw, Seminole, Shawnee, Chickasaw and Kiowa hybrids. With the exception of one individual (¾ Pawnee–¼ Chinook), the three tribal samples show no biological affiliation with Western or Southwestern tribes. There were no Pawnee-Caddo in the present samples even though the Caddo are as geographically close to the Pawnee as are the Wichita.

TABLE 1. INFLUENCE ON PHENOTYPE FREQUENCIES BY INCLUSION OF CLOSE RELATIVES

Phenotypes	PAWNEE "Full Blood"				WICHITA "Full Blood"			
	Entire Sample		Sub-sample (maximum r. 125)		Entire Sample		Sub-sample (maximum r. 125)	
	No.	%	No.	%	No.	%	No.	%
O	45	44.6	23	50.0	29	46.8	10	43.5
A	54	53.5	21	45.6	27	43.6	11	47.8
B	2	1.9	2	4.3	3	4.8	1	4.3
EB	0	0.0	0	0.0	3	4.8	1	4.3
Total	*101*		*46*		*62*		*23*	
MS	29	28.7	17	37.0	10	16.1	4	17.4
MNS	17	16.8	4	8.7	16	25.8	5	21.7
NS	1	1.0	1	2.1	8	12.9	2	8.7
Ms	33	32.8	15	32.6	13	21.0	3	13.0
MNs	20	19.8	8	17.4	11	17.7	7	30.4
Ns	1	1.0	1	2.1	4	6.4	2	8.7
Total	*101*		*46*		*62*		*23*	
CCDee	26	25.7	10	21.7	13	21.0	3	13.0
CcDEe	30	29.7	15	32.6	25	40.3	6	26.1
ccDEE	14	13.9	6	13.0	8	12.9	5	21.7
CcDee	7	6.9	2	4.3	7	11.3	3	13.0
ccDEe	8	7.9	5	10.1	6	9.7	4	17.4
CCDEe	6	5.9	3	6.4	3	4.8	2	8.7
CcDEE	9	8.9	4	8.7	0	0.0	0	0.0
ccDee	1	1.0	1	2.1	0	0.0	0	0.0
CCDEE	0	0.0	0	0.0	0	0.0	0	0.0
Total	*101*		*46*		*62*		*23*	
Fy(a+)	94	93.0	42	91.3	55	88.7	21	91.3
Fy(a−)	7	6.9	4	8.7	7	11.3	2	8.7
Total	*101*		*46*		*62*		*23*	
K+	4	4.0	2	4.4	0	0.0	0	0.0
K−	97	96.0	44	95.6	62	100.0	23	100.0
Total	*101*		*46*		*62*		*23*	
Le(a+)	0	0.0	0	0.0	0	0.0	0	0.0
Le(a−)	101	100.0	46	100.0	62	100.0	23	100.0
Total	*101*		*46*		*62*		*23*	
P+	95	94.0	44	95.6	50	80.6	19	82.6
P−	6	5.9	2	4.3	12	19.3	4	17.4
Total	*101*		*46*		*62*		*23*	

MATERIALS AND METHODS

The following sera were used: Anti-A and B—saline tube test, *Ulex europeus* lectin (anti-H)—saline tube test (Boyd, 1954), Anti-M and N—saline tube test, Anti-D—albumin rapid tube test, Anti-D—saline capillary test (Chown, 1944), (All saline capillary tests were run by the technique of Chown, (1944).) Anti-C—albumin rapid tube test, Anti-C—saline capillary test, Anti-c—albumin rapid tube test, Anti-E—albumin rapid tube test, Anti-E—saline capillary test, Anti-e—albumin rapid tube test, Anti-Fy^a—Coombs test, Anti-Kell—Enzyme capillary test (Gray, 1959), Anti-Le^a—saline tube test, Anti-P—saline tube test, and Anti-S—saline capillary test.

Blood samples were drawn from the ear into saline and tested within 12 hours with the samples made up in the diluent appropriate to each serum. Positive and negative reactions were checked against a cell panel obtained from Knickerbocker Biosales. One example of a weak D antigen was found using the Chown capillary method. This was not termed a D^u since the tube reaction using albumin anti-D was within the normal range of agglutination.

RESULTS

The observed and expected phenotype frequencies are listed in Table 2. Gene frequencies are listed in Table 3. ABO frequencies were computed by Bernstein's formulae and adjusted by Bernstein's correction. The variances and standard errors were derived using the formulae presented in Boyd (1956). The MNS and Rh gene frequencies were estimated by maximum likelihood methods derived by Boyd (1954) and Boyd (1958) respectively. Gene frequencies were not computed for the Duffy, Kell, Lewis and P groups since only one anti-serum was available for each system and each of these systems is known to consist of multiple alleles.

In spite of the small numbers in the Caddo and Wichita "full blood" sub-samples, the internal consistency for ABO and Rh groups is good. No definite reason can be offered as to why the Pawnee MNS observed frequencies diverge the most from expectation as the Pawnee sub-samples were the largest of the three groups tested.

Since the anthropologist is beset with sampling problems due to population size and structure, it is advisable to use efficient estimates of gene frequencies (Table 4). The Rh frequencies for the two Pawnee sub-samples are not greatly altered by the use of maximum likelihood estimates; however, considerable change in frequencies is apparent for the two Wichita sub-samples. The reverse situation is true for the MNS frequencies. Two rounds of maximum likelihood calculations produce significant changes in the Pawnee MNS estimates.

DISCUSSION

Limiting factors influence the tracing of prehistoric migrations of inter-tribal affiliations of American Indians by the comparison of blood group gene frequencies. The Pawnee and Wichita suffered severe reduction in numbers over a

TABLE 2. BLOOD GROUP PHENOTYPE FREQUENCIES OF PAWNEE, WICHITA AND CADDO INDIANS

Phenotypes	PAWNEE						WICHITA						CADDO					
	"Full Blood"			Tribal Status			"Full Blood"			Tribal Status			"Full Blood"			Tribal Status		
	% Obs.	N Obs.	N Exp.	% Obs.	N Obs.	N Exp.	% Obs.	N Obs.	N Exp.	% Obs.	N Obs.	N Exp.	% Obs.	N Obs.	N Exp.	% Obs.	N Obs.	N Exp.
ABO																		
O	50.0	23	23.4	57.5	46	46.4	43.5	10	9.7	55.1	27	26.5	94.4	17	17.0	70.2	33	33.0
A	45.6	21	20.5	40.0	32	31.6	47.8	11	11.3	40.8	20	20.4	5.6	1	1.0	29.7	14	14.0
B	4.3	2	1.5	2.5	2	1.5	4.3	1	1.4	2.0	1	1.5	0.0	0	0.0	0.0	0	0.0
AB	0.0	0	0.5	0.0	0	0.5	4.3	1	0.6	2.0	1	0.5	0.0	0	0.0	0.0	0	0.0
Total		*46*	*45.9*		*80*	*80.0*		*23*	*23.0*		*49*	*48.9*		*18*	*18.0*		*47*	*47.0*
MNS																		
MS	36.9	17	8.4	30.0	24	17.0	17.4	4	4.0	18.4	9	7.3	22.2	4	4.1	31.9	15	15.4
MNS	8.7	4	5.0	12.5	10	9.6	21.7	5	5.4	18.4	9	9.2	11.1	2	1.8	12.8	6	5.2
NS	2.2	1	0.7	1.2	1	1.1	8.7	2	1.8	6.1	3	2.8	0.0	0	0.1	0.0	0	0.3
Ms	32.6	15	22.5	38.8	31	38.22	13.0	3	3.3	22.4	11	13.6	27.8	5	6.0	29.8	14	15.4
MNs	17.4	8	8.4	16.2	13	12.9	30.4	7	5.8	30.6	15	12.9	38.9	7	5.0	25.5	12	9.3
Ns	2.2	1	0.9	1.2	1	1.1	8.7	2	2.6	4.0	2	3.1	0.0	0	1.0	0.0	0	1.4
Total		*46*	*45.9*		*80*	*79.9*		*23*	*22.9*		*49*	*48.9*		*18*	*18.0*		*47*	*47.0*
Rh																		
CCDee	21.7	10	8.4	23.8	19	16.3	13.0	3	3.0	16.3	8	8.7	27.8	5	4.7	29.8	14	12.9
CcDEe	32.6	15	15.8	35.0	28	29.8	26.1	6	7.5	38.8	19	17.6	22.2	4	4.5	31.9	15	15.0
ccDEE	13.0	6	6.7	15.0	12	12.9	21.7	5	4.2	16.3	8	8.3	5.8	1	0.8	6.4	3	3.9
CcDee	4.4	2	4.1	3.8	3	6.6	13.0	3	2.7	8.2	4	4.9	11.1	2	2.9	6.4	3	5.1
ccDEe	10.9	5	3.8	11.2	9	5.9	17.4	4	3.1	12.2	6	4.8	5.5	1	1.1	8.5	4	2.8
CCDEe	6.5	3	3.3	5.0	4	4.0	8.7	2	0.8	6.1	3	1.9	16.6	3	2.3	8.5	4	4.1
CcDEE	8.7	4	3.0	5.0	4	3.5	0.0	0	1.0	2.0	1	1.9	5.5	1	0.9	6.4	3	2.3
ccDee	2.1	1	0.5	1.2	1	0.7	0.0	0	0.6	0.0	0	0.7	5.5	1	0.4	2.1	1	0.5
CCDEE	0.0	0	0.3	0.0	0	0.2	0.0	0	0.0	0.0	0	0.1	0.0	0	0.3	0.0	0	0.3
Total		*46*	*45.9*		*80*	*79.9*		*23*	*22.9*		*49*	*48.9*		*18*	*17.9*		*47*	*46.9*
Duffy																		
Fy(a+)	91.3	42		87.5	70		91.3	21		91.8	45		77.7	14		85.1	40	
Fy(a−)	8.7	4		12.5	10		8.7	2		8.1	4		22.2	4		14.8	7	
Total		*46*			*80*			*23*			*49*			*18*			*47*	
Kell																		
K+	4.3	2		3.7	3		0.0	0		0.0	0		0.0	0		0.0	0	
K−	95.6	44		96.25	77		100.0	23		100.0	49		100.0	18		100.0	47	
Total		*46*			*80*			*23*			*49*			*18*			*47*	
P																		
P+	95.6	44		95.0	76		82.61	19		79.6	39		77.8	14		79.5	30	
P−	4.3	2		5.0	4		17.39	4		20.4	10		22.2	4		20.5	8	
Total		*46*			*80*			*23*			*49*			*18*			*47*	
Lewis																		
Le(a+)	0.0	0		0.0	0		0.0	0		0.0	0		0.0	0		0.0	0	
Le(a−)	100.0	46		100.0	80		100.0	23		100.0	49		100.0	18		100.0	47	
Total		*46*			*80*			*23*			*49*			*18*			*47*	

short period of time followed by a gradual population increase so that the possibility of considerable genetic drift cannot be discounted. Some tribes such as the Caddo, Kansa, Iowa and Missouri did not recover from the effects of white contact so that adequate samples can no longer be obtained. A sample of living

TABLE 3. ABO, MNS, AND RH GENE FREQUENCIES FOR PAWNEE, WICHITA AND CADDO INDIANS

Genes or Chromosomes	PAWNEE		WICHITA		CADDO	
	"Full Blood"	Tribal Sample	"Full Blood"	Tribal Sample	"Full Blood"	Tribal Sample
ABO	n = 46	n = 80	n = 23	n = 49	n = 18	n = 47
p	.264 ± .050	.226 ± .036	.307 ± .082	.243 ± .070	.029 ± .028	.162 ± .099
q	.022 ± .015	.012 ± .010	.044 ± .034	.020 ± .014	.000	.000
r	.714 ± .051	.762 ± .041	.649 ± .105	.736 ± .048	.972 ± .028	.838 ± .099
MNS						
mS	.128 ± .042	.140 ± .028	.185 ± .049	.126 ± .017	.174 ± .037	.237 ± .040
ms	.699 ± .050	.691 ± .037	.380 ± .065	.527 ± .042	.576 ± .096	.571 ± .047
nS	.044 ± .008	.052 ± .002	.101 ± .019	.096 ± .018	.009 ± .000	.018 ± .000
ns	.129 ± .029	.117 ± .021	.333 ± .057	.251 ± .036	.241 ± .038	.174 ± .025
Rh						
CDe (R_1)	.426 ± .053	.451 ± .040	.363 ± .071	.422 ± .048	.513 ± .094	.523 ± .052
cDE (R_2)	.383 ± .051	.402 ± .040	.428 ± .072	.413 ± .033	.207 ± .075	.289 ± .048
cDe (R_o)	.106 ± .033	.092 ± .024	.159 ± .054	.118 ± .033	.154 ± .069	.104 ± .033
CDE (R_z)	.085 ± .030	.055 ± .017	.050 ± .033	.047 ± .017	.126 ± .070	.083 ± .052

TABLE 4. DIFFERENCE IN GENE FREQUENCIES CALCULATED BY TWO METHODS

Genes or Chromosomes	PAWNEE				WICHITA			
	"Full Blood"		Tribal Status		"Full Blood"		Tribal Status	
	Mourant	M. L.	Mourant	M. L.	Mourant	M. L.	Mourant[1]	M. L.[2]
CDe (R_1)	.441	.426	.455	.451	.293	.363	.394	.422
cDE (R_2)	.398	.383	.405	.402	.358	.428	.394	.413
cDe (R_o)	.091	.106	.089	.092	.229	.159	.136	.118
CDE (R_z)	.070	.085	.051	.055	.120	.050	.076	.047
mS	.233	.128	.202	.140	.171	.185	.133	.126
ms	.593	.699	.630	.691	.394	.380	.520	.527
nS	.045	.044	.048	.052	.107	.101	.111	.096
ns	.129	.129	.120	.117	.328	.333	.236	.251

[1] Mourant, E. A. (1954).
[2] Boyd, W. C. (1954).

tribal members may not adequately represent the tribe of the same name in existence 500 years before. Tribal roles may list considerable numbers but the affiliation of an undetermined number may be cultural or political and not biological.

In the area under investigation, comparative blood group data for linguistically or geographically related tribes is not extensive so that little can be said concerning gene clines. Many of the earlier studies concentrated on areas instead of tribal background so that the frequencies may represent composite samples

TABLE 5. ABO AND RH ALLELE AND FY(A+) FREQUENCIES OF SEVERAL AMERICAN INDIAN TRIBES

Tribe or Area	Investigator	Number	Alleles										Phenotype % Fy(a+)
			ABO			Rh							
			p	q	r	R_1	R_2	R_z	R_0	r''	r'	r	
Blackfoot	Chown, 1953	39	.608	.000	.392								97.44
Blood	Chown, 1953	241	.582	.010	.407	.469	.401	.038	.000	.026	.000	.064	93.24
Stoney	Chown, 1955	155	.161	.000	.839	.484	.349	.019	.021	.029	.000	.097	88.39
Sarcee	Chown, 1955	95	.281	.000	.718								92.13
Chippewa	Matson, 1954	161	.064	.000	.936	.315	.587	.019	.000	.079	.000	.000	98.22
Navaho	Brown, 1958	106	.241	.000	.759	.375	.261	.026	.338	.000	.000	.000	
Apache	Brown, 1958	179	.234	.000	.766	.362	.264	.005	.369	.000	.000	.000	
Pima	Brown, 1958	489	.100	.001	.899	.444	.263	.051	.126	.035	.081	.000	2.72[1]
Cherokee Agency, N. C.	Snyder, 1926	250	.088	.041	.863								
Cherokee	Pollitzer, 1958	136	.018	.004	.978								
Choctaw-Chickasaw Sanatorium	Snyder, 1926	137	.048	.004	.947								
Ponca Agency Whiteage, Okla.	Snyder, 1926	100	.058	.016	.917								
Wichita Tribe	p[2]	49	.243	.020	.736	.422	.412	.047	.118	.000	.000	.000	91.84
Pawnee Tribe	p	80	.226	.012	.761	.451	.401	.055	.092	.000	.000	.000	87.50

[1] Sample size of 184.
[2] Present study.

of several tribes. In the only recent study, Pollitzer (1958) found 95% type O among full blood Cherokee living in North Carolina. It does appear that the Wichita and Pawnee have a much higher frequency of type A that other Southeastern tribes or tribes with Southeast cultural affiliation, which may indicate a northern Plains affiliation (Table 5) despite evidence to the contrary. Lowie (1954) believes that, on the basis of cultural and archaeological evidence, the Pawnee and Wichita can be connected with the Southeast and, in turn can be related linguistically to the Cherokee. The MNS distribution of the Pawnee and Wichita do not provide much in the way of comparative information since early studies did not include these factors and the present study shows some internal inconsistency. The frequency of R_o is higher than that observed for northern tribes (Brown, 1958). The Fy^a distribution is similar to that observed for northern tribes (Chown and Lewis, 1953, 1955; Matson, Koch and Levine, 1954) and shows little resemblance to that observed for the Southwest (Brown, Hannah, Dahlberg, and Strandskov, 1958). The few K+ individuals probably represent the result of white admixture even though this information does not appear in their family histories. Two of the three K+ individuals in the Pawnee tribal sample are first cousins. Two Le (a+) individuals were found in the

total series but both of these were less than 1/4 Indian and were not included in the sub-samples.

SUMMARY

Blood group gene frequency data are presented for the Caddo, Wichita and Pawnee Indians, all belonging to one linguistic stock and all located in Oklahoma. The non-random structure of small American Indian tribes is presented in the form of an inter-tribal genealogy. The effects of tribal structure on sampling and phenotype frequencies is discussed. Gene frequency estimates for MNS and Rh grounps are derived by both inefficient, and efficient maximum likelihood methods with the latter giving, in several instances, quite different estimates. While tribes belonging to the Caddoan linguistic stock are placed in the Southeastern culture area, their gene frequencies indicate a northern biological relationship.

ACKNOWLEDGMENTS

We wish to express our appreciation to Mr. James Sun Eagle, Chief of the Pawnee Tribe, Mr. Frank Miller, tribal leader of the Wichita Tribe, and Mr. Wilbur Williams, tribal leader of the Caddo Tribe for their assistance and cooperation in contacting tribal members and checking family histories. We are indebted to Dr. H. A. Hudgins, Area Medical Director, U. S. Public Health Service and his staff for their assistance and for providing laboratory facilities at Pawnee. Dr. Kenneth Richter and Dr. Alice Brues of the Department of Anatomy, University of Oklahoma Medical School provided laboratory facilities. Dr. Griffitts of the Dade Reagents, Inc., and Dr. Bruce Chown of the Rh Laboratory very kindly provided anti-sera. Appreciation is also expressed to Mrs. Rene Bock and Miss Sonja Homme for technical assistance in the field.

REFERENCES

Boyd, W. C. 1939. Blood Groups. *Tabulae Biologicae* **17: 113–240.**

Boyd, W. C., and Shapleigh, Elizabeth. **1954. Diagnosis of subgroups of blood groups A and AB by use of plant agglutinins (lectins).** ***J. Laborat. Clin. M.*** **44: 235–237.**

Boyd, W. C. 1954. Maximum likelihood method for estimation of gene frequencies from MNS data. *Am. J. Human Genet.* 6: 1–10.

Boyd, W. C. 1956. Variances of gene frequency estimates. *Am. J. Human Genet.* 8: 24–38.

Boyd, W. C. 1958. Gene frequency estimates for MNS data when four sera are used or for Rh data when five sera are used but all individuals are positive with Anti-D. (unpublished).

Brown, K. S., Hanna, B. L., Dahlberg, A. A., and Strandskov, H. H. 1958. The distribution of blood group alleles among Indians of southwest North America. *Am. J. Human Genet.* 10: 175–195.

Chown, B. 1944. A rapid, simple and economical method for Rh agglutination. *Am. J. Clin. Path.* 14: 114–115.

Chown, B., and Lewis, M. 1953. The ABO, MNSs, P, Rh, Lutheran, Kell, Lewis, Duffy and Kidd blood types and the secretor status of the Blackfeet Indians of Alberta, Canada. *Am. J. Phys. Anthrop.* 11: 369–383.

Chown, B., and Lewis, M. 1955. The blood group and secretor genes of the Stoney and Sarcee Indians of Alberta, Canada. *Am. J. Phys. Anthrop.* 13: 181–189.

Gray, M. P. 1959. Use of bromelin in blood typing. *Am. J. Laborat. Clin. M.* (in press).

Kroeber, A. L. 1947. *Cultural and Natural Areas of Native North America.* Berkeley, Calif.: Univ. California Press.

Lowie, R. H. 1954. *Indians of the Plains.* New York: McGraw-Hill.

Matson, G. A., Koch, E. A., and Levine, P. 1954. A study of the hereditary blood factors among the Chippewa Indians of Minnesota. *Am. J. Phys. Anthrop.* 12: 413–426.

Mourant, A. E. 1954. *Distribution of the Human Blood Groups.* Springfield, Ill.: C. C Thomas.

Pollitzer, W. S. 1958. (unpublished).

SYMPOSIUM
ON
Cytology and Cell Culture Genetics of Man[1]

Presented at University Park, Pennsylvania, on September 2, 1959

Edited by GORDON ALLEN
National Institute of Mental Health

[1] This symposium, financed by a grant (3M-9161) from the National Institute of Mental Health, U. S. Public Health Service, was held under the auspices of the American Institute of Biological Sciences and the sponsorship of the American Society of Human Genetics and the Genetics Society of America. The program was planned under Madge T. Macklin, President of the American Society of Human Genetics, by the Program Committee for the Society's 1959 Annual Meeting, Gordon Allen, Ray C. Anderson, and H. Eldon Sutton, Chairman.

PARTICIPANTS

H. BENTLEY GLASS, *Dept. of Biology, Johns Hopkins University, Baltimore, Maryland.* Chairman.

ERNEST H. Y. CHU, *Dept. of Botany, Yale University, New Haven, Connecticut.*

CHARLES E. FORD, *Medical Research Council, Radiobiological Research Unit, Harwell, England.*

MURRAY L. BARR, *Dept. of Microscopic Anatomy, University of Western Ontario, London, Canada.*

LEO SACHS, *Dept. of Experimental Biology, Weizmann Institute of Science, Rehovoth, Israel.*

ROBERT L. DEMARS, *Dept. of Medical Genetics, University of Wisconsin, Madison, Wisconsin.* (no manuscript received)

MARCO FRACCARO, *Institute of Medical Genetics, University of Uppsala, Sweden.*

CONTENTS

The Chromosome Complements of Human Somatic Cells[1]

ERNEST H. Y. CHU[2]

Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut

HISTORICAL

THE CHROMOSOME NUMBER OF MAN has been a question of unusual interest to biologists ever since it became known that these small deeply staining bodies are constant in number for a given species of animal or plant (cf. Stern, 1959). Arnold in 1879 published drawings of human tumor cells in division. Flemming in 1881–82 demonstrated mitosis from a corneal cell in which some twenty large and small, V, J and rod-shaped chromosomes were recognizable. The first attempt to determine the number of chromosomes in human cells was that by Hansemann, who, in 1891, reported three cells from "normal human tissues" with 18, 24 and more than 40 chromosomes, respectively. Since this early date, a number of other investigators, using both germinal and somatic tissues, have attempted to determine the exact number of chromosomes in man. Actual counts have ranged from 16 to 32, and there was no unanimity of opinion as to the true number. De Winiwarter in 1912 claimed that there were 47 chromosomes at metaphase in spermatogonia and 23 autosomal bivalents plus an unpaired X in primary spermatocytes. Wieman (1917) was the first to report the presence of XY chromosomes; Evans (1918) was the first to find a diploid number of 48 chromosomes in spermatogonia. In 1921 Painter reported in *Science* the presence of a small Y chromosome in males. In this same paper he stated that in spermatogonia "the counts range from 45 to 48 apparent chromosomes, although in the clearest equatorial plates so far studied only 46 chromosomes have been found." He went on to conclude that the diploid chromosome number in man is either 46 or 48. Two years later, Painter came to the conclusion that the correct diploid number was 48 in both sexes. It is interesting to note, however, that the materials used in his studies were testicular biopsies from three (two Negroes and one White) insane individuals. Whether or not chromosomal variations were present in these individuals was difficult to determine. In this same paper, noting

[1] Part of a Symposium on Cytology in Human Genetics held on Sept. 2, 1959, under the joint sponsorship of the American Society of Human Genetics and the Genetics Society of America during the meetings of the American Institute of Biological Sciences at Pennsylvania State University, August 30 to Sept. 3, 1959.

Some of the original research reported in this paper has been supported in part under a contract, AT(30-1)-1908, administered by N. H. Giles, with the U. S. Atomic Energy Commission.

[2] Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

the occurrence of giant or tetraploid spermatogonial cells, Painter suggested the possibility of an XXY hemaphrodite originating in a manner similar to the findings by Bridges (1922) in which XXY flies were derived from triploid *Drosophila*.

Painter's conclusion that the diploid chromosome number of man is 48 was supported by most authors in the following decades. However, de Winiwarter and Oguma (1926) adhered to the view that there was only a single sex-chromosome in spermatogonia. Koller's (1937) account of the behavior of the sex chromosomes during spermatogenesis conclusively proved the existence of the XY sex chromosome condition. From then on, the value of $2n = 48$ was generally accepted.

Early in 1956, Tjio and Levan made the surprising announcement that consistent counts of 46 chromosomes were obtained in lung fibroblast-like cell cultures established from four aborted Swedish embryos. In the same year, their counts were confirmed by Ford and Hamerton in testicular preparations from three British individuals.

An earlier solution to the question was probably prevented by problems of technical nature. Cytologists had been greatly handicapped in the study of mammalian and avian chromosomes by the fact that most species possess a large number of small chromosomes which usually crowd the metaphase plate and make counting and observation of individual chromosomes very difficult. With the exception of de Winiwarter, practically all early investigators working upon the spermatogenesis of man have used stale tissue—such as that obtained from executed criminals—in which the testis had remained in the body for some time after death. Painter (1923) stressed the cytological advantages of using freshly biopsied materials which were immediately fixed. Evans and Swezy (1929) expressed a similar view by pointing out that postmortem changes could alter the chromosome picture, giving rise to clumping, within ten or fifteen minutes between cessation of heart beat and autopsy. A determined effort was made by them to reduce the time intervals between death and fixation of cells. Their finest results came from cases in which testicular fragments were removed at the foot of the gallows and fixed in less than one minute. Nevertheless, the drawings of the best metaphase plates in these studies still show a considerable chromosome crowding and overlapping. A similar situation existed in several earlier studies in which adult somatic and embryonic tissues were used. However, the remarkable results obtained by these earlier investigators under the circumstances existing at the time command admiration and respect.

Before describing the number and morphology of human metaphase chromosomes in the light of modern studies, which are based primarily on somatic cells, the importance of studying chromosome morphology and behavior in spermatogenesis should be emphasized. The classical studies of de Winiwarter, Painter, Evans and Swezy, Koller, and others in establishing metaphase morphology of various chromosomes, in analyzing the sex chromosomes, and in following meiotic chromosome behavior certainly represent major triumphs of human cytology. The chromosomes in spermatogonia and spermatocytes are im-

portant as sources for comparison and verification. In addition, in view of the fact that the structure of metaphase chromosomes has only limited use for the detail required for a cytogenetic map, studies on pachytene chromosome structure such as those initiated by Schultz and St. Lawrence (1949) and followed by Yerganian (1957) and Kodani (1957a) should be actively pursued.

RECENT TECHNICAL ADVANCES IN THE STUDY OF HUMAN CHROMOSOMES

The rapid development of tissue and cell culture techniques in the past two decades has stimulated an experimental attack on many fundamental and applied problems of cell biology. The utilization of cell culture techniques also provides a number of advantages for cytological studies. For example, cell cultures present extremely favorable conditions for direct observation and photography of cells in the living state. The fact that the growth zone often consists of only a single layer of cells facilitates experimental treatments as well as cytological fixation and staining. Mitotic activity is usually more enhanced *in vitro* than *in vivo*, and it can be subjected to experimental control. Cell cultures also have a great advantage over sectioned histological preparations, which were used exclusively in earlier studies of human chromosomes, in that the cells in culture are more flattened and stretched on the substrate and no cellular material is lost or added by sectioning. Furthermore, cell culture techniques make it possible to compare chromosome constitutions of various tissues of the same individual, particularly useful in connection with the problem of somatic mosaicism. One possible criticism of the use of cultured cells for chromosome studies may be the known phenomenon of karyotypic changes which occur during growth *in vitro* (cf. Hsu, 1959). However, it is now possible to maintain a euploid condition of human cell lines for a considerable length of time without obvious chromosomal alterations (Tjio and Puck, 1958a; Chu and Giles, 1959a). Moreover, in the case of short-term cultures, such as those of bone marrow cells, this question does not arise.

As early as 1929, Kemp, among others, had used tissue cultures of human embryonic heart, liver and spleen to study chromosomes. Unfortunately, this approach was for many years almost completely neglected by cytologists. Similarly, the effect of a hypotonic medium in spreading chromosomes was not realized until 1952 when Hsu and Hughes accidentally and independently rediscovered this simple and very useful technique, which had been noted earlier by Eleanor Slifer (1934) and Margaret Lewis (1934). The favorable results thus obtained have since stimulated a great number of investigations on the chromosome cytology of mammalian and avian species. This technical advance also led Tjio and Levan to the finding of the new chromosome number in man, thus opening the modern reinvestigation of the subject.

The prime difficulty encountered in human cytology in the past has been the scarcity of controlled material and its capricious availability. This is particularly the case when surgical procedures are involved. While the source of material for study of human spermatogenesis is still limited, somatic cells offer a ready and favorable source of materials for chromosome and other studies. Tech-

niques are now available for short or long term cultivation of human cells of different origin derived by biopsy or autopsy from both adult and young. The development of culture techniques for chromosome analysis by Ford and his associates (1958), who have used human bone marrow cells; by Puck, Cieciura and Robinson (1958), Lejeune, Gautier and Turpin (1959a,c), Fraccaro, Kaijser and Lindsten, Harnden (in press) and others, who have used tiny skin biopsies; and by Hungerford and co-workers (1959), who have used leukocytes from peripheral blood, are particularly noteworthy. It appears that not only is an extensive cytological survey of human populations possible, but concentrated investigations on members of families with certain particular genetic constitutions are also feasible.

THE KARYOTYPE OF MAN

Since 1956, the chromosome number of 46 has been found in testicular preparations and in cultures of normal tissues of different origins by a number of workers (Ford, Jacobs and Lajtha, 1958; Tjio and Puck, 1958a,b; Chu and Giles, 1959a). Chu and Giles (1959a) concluded that every pair of homologous chromosomes of the human complement can be individually recognized. Furthermore, statistical analyses indicate that homologous autosomes from cells of the same or different individuals do not differ significantly either in relative length or in centromere position. The only difference between chromosome complements of the two sexes resides in the sex chromosomes. There are no significant differences among the X chromosomes or among the Y chromosomes from different individuals.

An idiogram of the human karyotype based on the results of chromosome measurement has been presented (Fig. 1). The autosomes, designated in Arabic numerals, of the human haploid chromosome complement are arranged in order of decreasing total length and of relative centromere positions. If two chromosomes are of equal length, the one having the more nearly median centromere is placed first. The sex chromosome pair, X and Y, is placed at the end. This

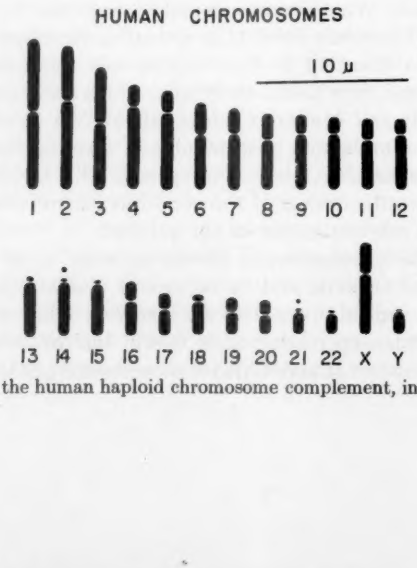

FIG. 1. Idiogram of the human haploid chromosome complement, including the sex pair.

system is adopted because it is the simplest and the least arbitrary. This idiogram is in almost complete agreement in cytological detail with those independently proposed by Tjio and Puck (1958b), by Ford, Jacobs, and Lajtha (1958), by Lejeune, Turpin, and Gautier (1959b), by Böök, Fraccaro, and Lindsten (1959), and others. The only major difference is in the systems employed in numbering the chromosomes. The need for a unified system to avoid confusion and to serve as a working basis is apparent. Hope was generally expressed, during recent discussions with a number of these workers, that a uniform nomenclature will soon be adopted.

The total number of cases in which the diploid number 46 has been recorded is now well over 200. There remain, however, the reports by Kodani (1957a, b; 1958a, b) of supernumerary chromosomes in man resulting in chromosome counts of 46, 47 and 48. Four possible explanations of these results, which are in disagreement with all other recent findings, may be considered. Firstly, the possibility of technical error cannot be dismissed. Secondly, on the basis of Kodani's observations, the basic 46 chromosomes, recognizable by their size and shapes, are common to all individuals. In those with one or two supernumeraries, no multiple chromosome association has been found. These facts seem to rule out the explanation of the origin of chromosome polymorphism by a Robertsonian type of chromosome evolution. By the same token, the lack of homology between these supernumeraries and any chromosomes of the basic set, and the absence of multivalent association in meiosis, make the explanation of the extra chromosomes as trisomics or tetrasomics unlikely.

Thirdly, despite the absence of any known cases in mammals, there exists a remote possibility of somatic elimination of supernumeraries, which would prevent their detection in individuals from whom somatic cells alone have been studied. However, the diploid number of 46 has been repeatedly found in primary spermatocytes and spermatogonial cells by Ford and associates (1958). In cases where both somatic and germinal tissues of the same individuals have been examined, there is no evidence of chromosome elimination (cf. Chu and Giles, 1959a). Examination of a number of embryonic tissues has also failed to show any evidence of chromosome elimination, even at the early stages of development (Chu and Giles, 1959a and unpublished).

The fourth possible explanation of Kodani's results may be that differences exist in various human populations. According to his observations, supernumeraries seem to occur with a much higher frequency in certain Oriental populations, and, in one instance, supernumeraries were found in a Caucasian individual (Kodani, 1958b). It would be interesting to examine the somatic chromosomes of those reported to have supernumeraries. Additional independent examinations of individuals from these populations is highly desirable in order to clarify this point. Recently, Makino and Sasaki (1959) reported six Japanese cases in which cultured embryonic cells were used, all showing 46 somatic chromosomes.

On the basis of present overall evidence, it is reasonable to conclude that 46 is the correct basic diploid chromosome number in man. The author wishes to stress the point that there is a great degree of constancy, in both chromosome

number and morphology, of this basic set in normal individuals from the different human populations which have been studied so far. This information is essential for the analysis of radiation-induced human chromosome aberrations (Chu and Giles, 1959b), as well as for the study of naturally occurring human chromosome variations. On the other hand, it should also be kept in mind that chromosome studies in man are just beginning, and variations in number as well as in morphology and structure are to be expected here as in other more thoroughly studied species. Indeed, in this year, there have been significant discoveries of some most interesting chromosomal variations found in individuals with various hereditary conditions. The genetical and clinical implications of these will be explored by the next speaker, who is among those responsible for these discoveries.

REFERENCES

Arnold, J. 1879. Beobachtungen über Kerntheilungen in den Zellen der Geschwülste. *Virchow's Arch.* 78: 279–301.

Böök, J. A., Fraccaro, M. and Lindsten, J. 1959. Cytogenetical observations in mongolism. *Acta Paediat.* 48: 453–468.

Bridges, C. B. 1922. The origin of variations in sexual and sex-limited characters. *Am. Natur.* 56: 51–63.

Chu, E. H. Y. and Giles, N. H. 1959a. Human chromosome complements in normal somatic cells in culture. *Am. J. Human Genet.* 11: 63–79.

Chu, E. H. Y. and Giles, N. H. 1959b. Qualitative and quantitative analyses of X-ray induced human chromosome aberrations in cultures of normal diploid somatic cells. (Abstract). *Genetics* 44: 503–504.

de Winiwarter, H. 1912. Études sur la spermatogenèse humaine. (I. Cellule de Sertoli. II. Hétérochromosome et mitoses de l'épithélium seminal). *Arch. Biol.* 27: 91–190.

de Winiwarter, H. and Oguma, K. 1926. Nouvelles recherches sur la spermatogenèse humaine. *Arch. Biol.* 36: 99–166.

Evans, H. M. 1918. *In* Babcock, E. B. and Clausen, R. E., *Genetics in Relation to Agriculture*, McGraw-Hill, p. 538, footnote.

Evans, H. M. and Swezy, O. 1929. The chromosomes in man, sex and somatic. *Mem. Univ. Calif.* 9: 1–65.

Flemming, W. 1882. Beiträge zur Kenntniss der Zelle und ihrer Lebenserscheinungen. Part III. *Arch. mikr. Anat.* 20: 1–86.

Ford, C. E. and Hamerton, J. L. 1956. The chromosomes of man. *Nature* 178: 1020–1023.

Ford, C. E., Jacobs, P. A., and Lajtha, L. G. 1958. Human somatic chromosomes. *Nature* 181: 1565–1568.

Hansemann, D. 1891. Ueber pathologische Mitosen. *Virchow's Arch.* 123: 356–370.

Harnden, D. G. (in press). *Brit. J. Exp. Path.*

Hsu, T. C. 1952. Mammalian chromosomes *in vitro*. I. The karyotype of man. *J. Hered.* 43: 167–172.

Hsu, T. C. 1959. Numerical variation of chromosomes in higher animals. *16th Growth Symposium: Developmental Cytology.* Dorothea Rudnick, ed. Ronald Press. pp. 47–62.

Hughes, A. 1952. Some effects of abnormal tonicity on dividing cells in chick tissue cultures. *Q. J. Micr. Sc.* 93: 207–220.

Hungerford, D. A., Donnelly, A. J., Nowell, P. C. and Beck, S. 1959. The chromosome constitution of a human phenotypic intersex. *Am. J. Human Genet.* 11: 215–236.

Kemp, T. 1930. Über die somatischen Mitosen bei Menschen und warmblütigen Tieren unter normalen und pathologischen Verhältnissen. *Zschr. Zellforsch.* 11: 429–444.

Kodani, M. 1957a. The karyotype of man with the diploid chromosome number of 48. *Proc. Internat. Genetics Symposia*, 1956, Tokyo and Kyoto, Japan, pp. 103–107.

KODANI, M. 1957b. Three diploid chromosome numbers of man. *Proc. Nat. Acad. Sc.* **43**: **285–292.**

KODANI, M. 1958a. Three chromosome numbers in Whites and Japanese. *Science* **127**: **1339–1340.**

KODANI, M. 1958b. The supernumerary chromosomes of man. *Am. J. Human Genet.* **10**: **125–140.**

KOLLER, P. C. 1937. The genetical and mechanical properties of sex chromosomes. III. Man. *Proc. R. Soc. Edinburgh* 57: 194–214.

LEJEUNE, J., GAUTIER, M. AND TURPIN, R. 1959a. Les chromosomes humaines en culture de tissus. *C. Rend. Acad. Sc.* **248**: **602–603.**

LEJEUNE, J., TURPIN, R. AND GAUTIER, M. 1959b. Le mongolisme, maladie chromosomique (trisomie). *Bull. Acad. Nat. Méd.* **143**: **256–265.**

LEJEUNE, J., TURPIN, R. AND GAUTIER, M. 1959c. Le mongolisme, premier exemple d'aberration autosomique humaine. *Ann. Génét.* 1: **41–49.**

LEWIS, M. R. 1934. Reversible solation of the mitotic spindle of living chick embryo cells studied *in vitro. Arch. exp. Zellforsch.* **16**: **159–166.**

MAKINO, S. AND SASAKI, M. 1959. On the chromosome number of man. *Proc. Japan Acad.* **35**: **99–104.**

PAINTER, T. S. 1921. The Y chromosome in mammals. *Science* **53**: **503–504.**

PAINTER, T. S. 1923. Studies in mammalian spermatogenesis. II. The spermatogenesis of man. *J. Exp. Zool.* **37**: **291–334.**

PUCK, T. T., CIECIURA, S. J., AND ROBINSON, A. 1958. Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. *J. Exp. M.* **108**: **945–956.**

SCHULTZ, J. AND ST. LAWRENCE, P. 1949. A cytological basis for a map of the nucleolar chromosome in man. *J. Hered.* **40**: **31–38.**

SLIFER, E. H. 1934. Insect Development. VI. The behavior of grasshopper embryos in anisotonic balanced salt solutions. *J. Exp. Zool.* **67**: **137–157.**

STERN, C. 1959. The chromosomes of man. *J. M. Educ.* **34**: **301–314.**

TJIO, J. H. AND LEVAN, A. 1956. The chromosome number of man. *Hereditas* **42**: **1–6.**

TJIO, J. H. AND PUCK, T. T. 1958a. Genetics of somatic mammalian cells. II. Chromosomal constitution of cells in tissue culture. *J. Exp. M.* **108**: **259–268.**

TJIO, J. H. AND PUCK, T. T. 1958b. The somatic chromosomes of man. *Proc. Nat. Acad. Sc.* **44**: **1229–1237.**

WIEMAN, H. L. 1917. The chromosomes of human spermatocytes. *Am. J. Anat.* **21**: **1–22.**

YERGANIAN, G. 1957. Cytologic maps of some isolated human pachytene chromosomes. *Am. J. Human Genet.* 9: 42–54.

Human Cytogenetics: Its Present Place and Future Possibilities[1]

C. E. FORD

Medical Research Council, Radiobiological Research Unit, Harwell, England

THE NUMBER OF HUMAN CHROMOSOMES

I THINK IT WOULD BE FAIR to say that four years ago none would have foreseen the developments in human cytogenetics that have actually taken place. My own attitude at that time may have been typical. We had a visit one day from an Oxford surgeon, Mr. Moloney, who showed interest in the squash preparations of seminiferous tubules we were making at that time from Carter's mouse translocation stocks, and offered to provide us with human material. I thanked him, of course, and the thought that ran through my mind may have been something like this, "It would be interesting to see human chromosomes when there is a little less pressure of work, but everyone knows that the diploid number is 48 and there is probably very little more we can learn at present."

The following year I read with an amazement that I am sure I must have shared with many others, Tjio and Levan's short paper in Hereditas (1956), announcing their counts of 46 chromosomes in tissue cultures established from foetal lung. I immediately remembered Mr. Moloney's offer, and with his kind assistance, Hamerton and I (1956) were quickly able to confirm the new number by counting 23 bivalents regularly at first spermatocyte metaphase. Our work was therefore inspired solely by Tjio and Levan's discovery: it was in no sense independent.

The surprise had hardly subsided when Kodani's first paper (1957) appeared bringing an apparent reprieve for the number 48; an indication that the number might vary, at least among Japanese; the suggestion that the chromosomes in excess of 46 were inert supernumeraries; and the implication that human populations might differ from one another in respect of these additional chromosomes. Since then however, notwithstanding a further, confirmatory contribution from Kodani (1958), the evidence in favour of the diploid number 46, at least in persons of European ancestry, has continued to mount; (Bender 1957; Hsu et al., 1957; Ford, Jacobs and Lajtha, 1958; Tjio and Puck, 1958; Lejeune, Gautier and Turpin, 1959; Chu and Giles, 1959). Published evidence regarding the chromosomes of other ethnic groups is still very meagre, but there is a recent short paper by Makino and Sasaki (1959) reporting counts of 46 in tissue cultures established from six Japanese foetuses, and Professor Makino has kindly al-

[1] Part of a Symposium on Cytology in Human Genetics held on Sept. 2, 1959, under the joint sponsorship of the American Society of Human Genetics and the Genetics Society of America during the meetings of the American Institute of Biological Sciences at Pennsylvania State University, August 30 to Sept. 3, 1959.

lowed me to say that he and his colleagues have now made counts on tissue cultures from a total of 39 different Japanese foetuses and that in every one they find the diploid number 46. Taken at face value these observations are not reconcilable with Kodani's. But it must be remembered that Kodani's observations were made on testicular material, and until there is independent examination of the meiotic chromosomes Stern's suggestion (1959a) that supernumerary chromosomes might be confined to the germ tract cannot be rejected.

HUMAN CHROMOSOME MORPHOLOGY

Within the last 15 months idiograms of human somatic chromosomes have been published by three groups. Our own (Ford, Jacobs and Lajtha, 1958) was based on measurements of the chromosomes in 3 bone marrow mitoses and was not meant to be other than a guide for further work. Nevertheless there is a considerable measure of agreement between this and those of Tjio and Puck (1958) and Chu and Giles (1959) which were prepared from measurements of chromosomes in tissue-cultured cells. Such small differences as there are may well reflect differences in contraction of whole chromosomes or chromosomic arms brought about by differences of technique or in origin of the cells examined. Nevertheless we should bear in mind the possibility of true, structural polymorphism of the human chromosomes, perhaps particularly of the X and Y. Comparison of the chromosome sets of different ethnic groups immediately suggests itself as the most likely method of revealing polymorphism if it exists. It is unnecessary to stress the interest for anthropology if any form of chromosome polymorphism should be revealed.

ABNORMALITIES OF SEX-CHROMOSOME CONSTITUTION

Tjio and Puck (1958) on the one hand and Chu and Giles (1959) on the other claimed to be able to identify both X and Y as individual chromosomes. We originally were only able to define Y as one of 5 small acrocentric chromosomes in the male, and the X as one of 15 medium sized metacentric chromosomes (Ford et al. 1958). Nevertheless this was sufficient to justify beginning an investigation of the chromosomes in patients exhibiting anomalies of sex development. Now we can frequently identify Y as an individual chromosome in our marrow preparations and the X as one of 5, sometimes 3.

Interest in these anomalies had been greatly stimulated by the discovery of 'sex chromatin' by Barr and Bertram (1949) and by the interpretation placed on it, namely that it represented fused heterochromatic regions of the two X chromosomes. It was soon found that the essentially male patients with Klinefelter's Syndrome, or seminiferous tubule dysgenesis, mostly exhibited sex chromatin in buccal smears, or skin biopsy nuclei (Bradbury et al., 1956; Plunkett and Barr, 1956). The essentially female patients with gonadal dysgenesis (the more extreme forms of which are known as Turner's Syndrome) were mostly chromatin negative (Decourt et al., 1954; Polani et al., 1954; Wilkins et al., 1954). There were suggestions that these two classes of patient, in which the sex chromatin diagnosis did not conform with the apparent sex, might represent examples of reversal of the genetic or chromosomal sex.

In February, 1958, Miss Jacobs and I, in conjunction with Dr. W. M. Davidson and Dr. D. R. Robertson Smith examined the chromosomes of a chromatin-positive Klinefelter patient by the bone marrow method. The specimen was a poor one and we found only 5 reasonably 'good' cells in metaphase, all of which appeared to have the chromosomes of a normal female (Ford et al. 1958). It seemed as though those who spoke of 'reversal of genetic sex' might be right.

In the event this proved to be an unlucky observation. At the end of the year Jacobs and Strong (1959) and my associates and myself (1959) independently examined the chromosomes of two further chromatin-positive Klinefelter patients. The great majority of cells of both patients were recorded as containing 47 chromosomes, which were interpreted to include, almost certainly, two X chromosomes and a Y. The marrow specimen from our patient also contained a number of cells with 46 chromosomes and appeared to be a mosaic, as far as his marrow was concerned, of XXY cells and XX cells.

Subsequently we have examined the chromosomes of a further 6 chromatin-positive Klinefelter patients, including the remarkable mongol Klinefelter case I shall mention again later. In every one there is very little doubt that the patient developed from an XXY zygote. The cytological observations relating to one of these patients were made by Dr. D. G. Harnden (in press) using tissue cultures established from skin explants on to plasma clots.

Further chromatin-positive Klinefelter patients have also been examined at Edinburgh and Lund. Dr. Levan and Dr. Nowakowski have kindly informed me of the work at Lund, which was carried out by Drs. Bergman and Reitalu on tissue cultures established from skin biopsies performed on two of Dr. Nowakowski's patients. Again 47-chromosome cells, interpreted to be XXY, predominated, although in cultures from one of the patients some 30 per cent of cells contained an additional acrocentric chromosome with no counterpart in the normal set. Our first (?XX) case appears to be, therefore, the one exception among some 12 examined. I have checked the original records and have no reason to suspect that they may have been wrong. Obviously we should endeavour to re-examine material from the same patient. In the light of the one mosaic marrow I have already mentioned and other examples I shall discuss shortly I don't think it is too fanciful to suggest mosaicism as a possible explanation.

The other group of patients in which 'reversal' of genetic sex had been suggested as a result of sex chromatin studies I shall refer to collectively as cases of Turner's Syndrome. We started with the knowledge that on the basis of colour blindness studies Polani, Lessof and Bishop (1956) had suggested that the chromatin-negative group might be XO: also that Danon and Sachs (1957) had found that in two patients observations on skin sections had not been consistent in respect of sex chromatin, indicating, they suggested, that these patients might be XO/XX chromosomal mosaics.

The first chromatin-negative Turner patient whose marrow we examined confirmed the suggestion made by Polani and his colleagues: virtually all the cells counted contained 45 chromosomes and all that were studied in detail were consistent with the XO interpretation.

Subsequently we have examined bone marrow preparations from three other cases of Turner's Syndrome, all in conjunction with Dr. Polani, Dr. Briggs and Dr. Almeida of Guy's Hospital, London. One of these was chromatin negative and two chromatin-positive, but all the preparations proved to contain mixtures of 45-chromosome cells and 46-chromosome cells. The last of the three gave preparations of good technical quality and 12 of the cells were analysed in detail. These detailed observations left little doubt that the 46-chromosome cells contained two X chromosomes and the 45-chromosome cells only one. We therefore interpret this case as an XO/XX mosaic, as foreshadowed by Danon and Sachs, and consider it probable that the other two examples mentioned are also XO/XX mosaics.

If this is so, then I suggest that it is likely that all three developed from XO zygotes. There is evidence that strong selective forces can operate and bring about differential proliferation of distinct clones of cells in the haemopoietic tissues of irradiated mice (Ford, Micklem and Gray 1959); also that there is selective elimination of cells with unbalanced chromosome sets in irradiated testis and bone marrow of another species, *Poterous tridactylus* (Sharman 1959). It would therefore not be surprising if an XX cell, arising by non-disjunction in an XO embryo, should be favoured and multiply differentially. On the other hand although an XO cell might arise by non-disjunctional loss of an X chromosome in an XX embryo, elimination or, at most, a slower rate of multiplication would seem to be a more likely fate. In any case I hope I have said enough to indicate that the evidence is not inconsistent with developments of all three mosaic patients from XO zygotes.

The chromosomes of several other chromatin-negative cases of Turner's Syndrome have now been examined by Fraccaro and his colleagues (1959), Tjio, Puck and Robinson (1959), and Harnden (in press) using tissue cultures; and by Jacobs and Stewart (unpublished) using the bone marrow method. All found cells with 45 chromosomes and agree in giving the XO interpretation.

THE CONSEQUENCES OF NON-DISJUNCTION

How do these abnormalities arise? The obvious answer is by non-disjunction during the meiotic divisions, and this is implicit in the references to XXY and XO zygotes I have made. (I ignore the rather unlikely possibility of somatic non-disjunction having produced a gonadal mosaic in one of the parents). Non-disjunction of the daughter X chromosomes in the first mitosis of an XY zygote would give XXY and YO daughter cells and as the latter is likely to be inviable an XXY individual could result. On the other hand, non-disjunction of daughter X chromosomes in the first mitosis of an XX zygote would give XXX and XO cells, and since there is no reason to suppose that the triple-X cell would be inviable, a nearly equal, perhaps bilateral, mosaic would be expected. Simple loss of one daughter X or Y at the first mitosis, or non-disjunction at a mitosis later than the first, would inevitably yield mosaics containing normal XX or XY cells as a component. I have already given reasons for supposing that the normal cells would dominate such partnerships, if the unbalanced cells survived at all.

We are therefore brought back to non-disjunction during oogenesis or spermatogenesis as being by far the most likely source of the anomalous XXY and XO individuals.

So far as I am aware there is no direct evidence of non-disjunction during the maturation of the gametes in man. However, Hamerton and I (1956) observed that the X and Y chromosomes were not associated at metaphase in several primary spermatocytes. Random assortment at anaphase should then lead to the formation of spermatid nuclei with *both* X and Y, and with *neither*. This could, and should, be checked by observations at second spermatocyte metaphase.

The results of Nowakowski, Lenz and Parada (1959) who found (inter alia) two instances of a colour-blind chromatin-positive Klinefelter son with non-colour blind parents, has been interpreted by Stern as showing evidence of non-disjunction in the maturation of the ova, either at the second division or (with recessive homozygosis) at the first division (1959). However, the possibility of non-disjunction at the first zygotic mitosis with doubling of the X chromosome carrying the colour-blindness gene received from a carrier mother is not rigorously excluded.

A similar argument can be put forward to explain the case described by Stewart (1959) of the colour blind XO Turner patient with a colour blind brother, non-colour blind father, and mother whose presumed heterozygosity was supported by anomaloscopic evidence of a minor defect. This case strongly suggests non-disjunction during spermatogenesis and fertilization by an O sperm, but again it does not provide rigorous proof, since non-disjunctional loss of a paternal X from a normally constituted XX zygote would give an XO cell, and we cannot be sure that its XXX partner would die and not contribute to an adult XXX/XO mosaic.

Although they fall short of full rigour, the cases just quoted do provide evidence in favour of non-disjunction at both oogenesis and spermatogenesis. But it would be wrong to fall into the error of associating non-disjunction at spermatogenesis with Turner's Syndrome and non-disjunction at oogenesis with Klinefelter's Syndrome. There is not the slightest reason to suspect that the complementary O ova and XY sperm would be any less viable than the XX ova and O sperm, for which the evidence for functional competence has just been given. It is merely that the XXY state permits one type of argument, the XO state another.

At a first examination we would expect non-disjunction of X and Y chromosomes at spermatogenesis to yield XY and O sperm in equal numbers, and consequently a one-to-one ratio of XXY and XO zygotes arising from this cause. Similarly, non-disjunction of the X-chromosome pair during maturation of the oocytes would lead to the formation of XX and O ova, and hence to the appearance of XXX, XXY, XO and YO zygotes, again in equal numbers. There should therefore be a near equality in the overall frequency of XXY and XO zygotes. However, the general experience seems to be that patients with Turner's syndrome are much less frequent than cases of Klinefelter's Syndrome, (Prader *et al.*, 1958) and this is supported by the wide disparity between the frequencies of

newborn apparent females that were chromatin-negative (none among 1804), and of newborn apparent males that were chromatin-positive (5 among 1911), as determined by Moore (1959). It is premature to consider in detail reasons for this discrepancy when the frequencies themselves are very imperfectly known, but obvious possibilities are, differential mortality of the zygotes *in utero*; differential maturation or fertilizing capacity of the alternative sperm types (compare Braden 1958); and possibly an inequality in the frequency of XX and O ova as a consequence of the asymmetry of the maturation divisions in the oocyte.

I have said that in addition to the XXY and XO zygotes, XXX and YO zygotes were to be expected. The first of these (XXX) has very recently been identified (Jacobs et al. 1959). The patient is a mentally sub-normal woman whose most interesting feature is the presence of *two* sex chromatin bodies in most of the nuclei seen in a buccal smear preparation. Formally the case is equivalent to the 'superfemale' *Drosophila* (Morgan, Bridges and Sturtevant 1925) but there is no exaggeration of secondary sexual characters in this patient; on the contrary, they are rather underdeveloped. YO has not been identified. As in *Drosophila* it may well be lethal to the embryo.

Apart from the intrinsic interest and medical importance of the anomalies of sex development I have just discussed, the new findings provide vital information regarding the roles of the sex chromosomes in sex determination. In the *Drosophila* system the X chromosomes promote development in the female direction and the autosomes in the male direction, whereas the Y is inert developmentally, though necessary for fertility (Morgan, Bridges and Sturtevant 1925). This system has long been used as a model and it has often been tacitly assumed that it would apply to mammals and man. In the event, however, it appears that the human system shows closer formal ties with that in the plant *Melandrium* (Westergaard 1940). XXY is a sterile male in man; in *Drosophila* it is a fertile female. The human XO is a sterile female; in *Drosophila* it is a sterile male.

The new evidence shows that the human Y chromosome strongly promotes development in the direction of the masculine phenotype. This is at once apparent when we compare XX (normal female) with XXY (sterile male), and XO (sterile female) with XY (normal male): addition of a Y chromosome converts a potentially female type into one that is essentially male. In this connection it is of the greatest interest that XO mice have now been identified by Welshons and Russell (1959). They are females and, it would appear, normally fertile. Evidently in the mouse also the Y chromosome is necessary for male development. We may come to find that this is generally true of mammals, notwithstanding the few species in which XO males have been claimed to occur (see Matthey, 1949).

THE MECHANISM OF SEX DETERMINATION

The condition known as testicular feminization also requires mention since suggestions have been made that it may trace to a gross chromosomal defect (Petterson and Bonnier 1937, Danon and Sachs 1957, Taillard and Prader 1957). A number of pedigrees showing hereditary transmission of this condition have

been published (see Taillard and Prader 1957). The striking features are that transmission is solely through the female sex and that there is a deficiency of normal males in the affected sibships. The affected individuals are essentially female, with well-developed secondary sexual characters, but absence or deficiency of axillary and pubic hair. The external genitalia are female, but atrophic testes are present. These are sometimes retained within the abdominal cavity but are more usually present in bilateral inguinal hernias. Sex chromatin is absent. Chu and Grumbach (unpublished) have examined the chromosomes of one patient exhibiting this condition and found no evident difference from those of a normal male. The same result was obtained independently by Harnden (in press) using skin cultures from one of two affected sisters. We must therefore reject gross chromosomal abnormality as a possible cause of this condition. It could be determined by a genetic sex-limited autosomal recessive or by a sex-linked recessive (Grumbach and Barr 1958). It might be possible to discriminate between these possibilities by relating the appearance of the condition to the inheritance of colour blindness in the affected families.

Recently Hungerford (1959) has reported briefly his finding of chromosomes indistinguishable from those of a normal female in cultures established from peripheral blood leucocytes of a true hermaphrodite, a Negro aged 12. He concludes "Thus cases of intersexuality in humans may involve mechanisms other than aberrations in the number of sex chromosomes". If the first word were omitted this statement would be quite unexceptionable. So much is evident from the cases of testicular feminization I have just discussed. But it is not a necessary conclusion from the evidence in his case since the very real possibility of mosaicism is not excluded.

Harnden (unpublished) has also examined the chromosomes of a true hermaphrodite in conjunction with Dr. C. N. Armstrong. Accounts of this patient have been published (Armstrong 1955, Armstrong et al. 1957). Harnden established cultures from skin biopsies taken from both sides of the body and each was divided into two, so that there were four cultures in all. The vast majority of cells in all cultures were apparently normal XX cells with 46 chromosomes, but in one of the cultures (only) there were some 20% of cells in which an abnormal chromosome was present, suggesting a reciprocal translocation. The change, it would seem, must have arisen during culture. In this case also the possibility of chromosomal mosaicism involving the gonads cannot be excluded. To get an undisputable solution to such problems it would be necessary to establish cultures from the organs most directly concerned, the gonads themselves.

CONGENITAL ABNORMALITIES

News that Lejeune, Gautier, and Turpin (1959a) in Paris had discovered an extra small acrocentric chromosome in tissue cultures established from several mongoloid imbeciles reached us at Harwell just 4 days before we were able to examine bone marrow preparations (in conjunction with Professor L. S. Penrose and his colleagues) from a remarkable patient who exhibited the stigmata

both of mongolism *and* Klinefelter's Syndrome (chromatin positive). We found that this patient had 48 chromosomes regularly, including an extra small acrocentric as well as the expected two X chromosomes and a Y (Ford et al. 1959). Independent confirmation that an extra chromosome was present in mongoloids also came at the same time from Edinburgh (Jacobs et al. 1959). Subsequently the French group added a further 4 cases to their original 6 (Lejeune et al. 1959b). Fraccaro has informed me that the group at Uppsala have also found the extra chromosome to be present in the two examples they have studied. There is thus a total of 19 cases of mongolism that have been examined without exception to the rule that an extra small acrocentric is present. Evidently, mongolism is a primary trisomic condition, the first that is viable to be identified in mammals. (Frankhauser and Humphrey (1950, 1954) obtained some trisomic larvae in the diploid by triploid *Ambystoma* crosses, but few survived long and all were malformed.)

We must suppose that autosomal non-disjunction at spermatogenesis or oogenesis is the most likely source of the additional chromosome, with a weighting in favour of oogenesis in view of the well-known maternal age effect in mongolism (Penrose 1954). The simple explanation of this relationship would then be that ageing increases the likelihood of non-disjunction during maturation of the ova. It has been claimed that the mothers of mongols as a group can be discriminated from other mothers by certain physiological tests (Ingvar Ek and Jensen 1959), and the authors assert that a genetic hypothesis alone is insufficient. Their conclusion is that "a certain combination between the genotype of the foetus, the endocrine disposition of the mother, and exogenous stress-releasing factors could be the pre-requisite for mongolism". Accumulated experience of trisomy in plants and *Drosophila* (triplo-IV) shows that each individual trisomic has its own characteristic phenotype, and it would be quite contrary to this experience to suppose that in certain circumstances the phenotype might not be expressed. A more likely explanation of the influence of maternal factors in mongolism would be through an increase in the chance of non-disjunction at oogenesis, *not* through direct influence on the development of the foetus. The latter could be expected to influence the degree of expression of the condition, but hardly whether or not it was expressed at all.

After the discovery that mongolism was a primary trisomic condition speculation inevitably ran to the possibility of further examples. Twenty-two different types are of course theoretically possible and it is likely that zygotes of all 22 kinds are formed from time to time. However, the inviability of triplo-II and triplo-III in *Drosophila melanogaster*, the failure of most trisomic *Ambystoma* larvae to survive for very long, and the fact that, notwithstanding the millions of laboratory mammals that have been bred, no primary trisomic has ever been reported, or (so far as I am aware) even suspected, makes it prudent to assume that most of the human primary trisomic zygotes would be inviable. It is significant that the extra chromosome of mongolism is one of the two smallest members of the human set and represents a duplication only of about

Table 1. Hereditary conditions that have been examined cytologically but in which no evidence of chromosomal abnormality has been found

Condition	Authority
Acrocephalosyndactyly	1, 3
Arachnodactyly (Marfan's Syndrome)	1, 2, 4
Chondrodystrophy	1
Crouzon's disease	1
Epiloia	3, 4
Gargoylism	1
Gaucher's disease	2
Hypopituitary dwarfism	4
Juvenile amaurotic idiocy	1
Laurence-Moon-Biedl syndrome	1
Little's disease	4
Osteogenesis imperfecta	1
Phenylketonuria	2

(1) Böök, Fraccaro and Lindsten (personal communication). (2) Tjio, Puck and Robinson (1959). (3) Harnden (unpublished). (4) Ford (unpublished).

1/200th part of the whole. Trisomy of the longest autosome would represent duplication of about 1/25th part and would therefore be much more likely to cause a serious disturbance of gene balance.

Nevertheless some of the 22 types might be viable and result in live births; even survive into adult life and breed. For each a characteristic phenotype would be expected, with (if fertile), hereditary transmission in a manner mimicking a dominant gene. A number of possible conditions have now been examined, but in none has any evident departure from the normal set of 46 chromosomes been found. In table I a list is given of those at present known to me, together with some recessive conditions that have been examined and also found, as expected, to have apparently normal chromosomes.

In addition to these, Harnden, Briggs and Stewart (1959) have reported apparently normal chromosomes in cultures from 4 anencephalic foetuses, and Harnden, in conjunction with Penrose, has found no evidence of chromosome abnormality in cultures established from a hydatidiform mole.

Lejeune and Turpin (unpublished) however, have now found a second example of autosomal abnormality and the first of a new type. In tissue cultures established from a boy with multiple skeletal defects the cells contained only 45 chromosomes, one of which was clearly abnormal. The missing chromosome was one of the two smallest acrocentric chromosomes and it appeared that the greater part of this chromosome had been translocated on to the short arm of one of the three *longer* acrocentrics. Since precisely the same altered chromosome was identified in several independently established cultures there can be no question, in this instance, of origin during culture. Nevertheless the nature of this change suggests that it is a unique one and unlikely to be found again, except perhaps in other members of the same family. On the other hand there may be many more instances of multiple congenital abnormalities associated with different, but again unique, chromosome rearrangements awaiting discovery.

LEUKAEMIA

This is not the place to go into the problem of the relationship of chromosomal changes in neoplastic cells to the mechanism of carcinogenesis. However, the study of normal somatic and neoplastic cell populations is a legitimate field for cytogenetics and the bone marrow method was originally developed with the object of examining the chromosomes of human leukaemic cells (Ford et al. 1958). This was prompted by the discovery that a very high proportion of independently arising primary reticular neoplasms ("leukaemias") of the mouse consisted of mixed populations of cells differing both from the normal and from one another in respect of one or more of four cytogenetic features. 1) Variation in the chromosome count as opposed to virtual constancy in the normal reticular tissues. 2) Increase in the mode of the chromosome-count distribution from the normal 40 to a value in the range 41 to 50. 3) Presence of one or more morphologically altered 'marker' chromosomes as a constant, and sometimes highly individual, character. 4) Presence of primary structural changes in the chromosomes of some cells (Ford et al. 1958, Ford and Mole 1959).

In conjunction with Dr. L. G. Lajtha we have now examined the chromosomes in sternal marrow cells of ten leukaemic patients. Three of these cases were mentioned briefly in our original work on somatic chromosomes (Ford et al. 1959b). In one of them the modal count was 45 and included a minute fragment. Two of the remaining seven cases were clearly abnormal. In one of them the modal count was 48; in the other the modal count was 46 including a minute fragment. We have recently recorded instances of spontaneous tumours of the Chinese hamster in which the chromosome count was either always, or almost always 22 (the diploid number of the species), but in which one or more characteristic morphologically altered chromosomes were present (Unpublished work). It is therefore necessary to be alert to the possibility of similar instances appearing among the human leukaemias. Needless to say the definition of such changes will inevitably be much easier in the hamster with its much lower number of chromosomes. To identify similar changes in the chromosomes of human marrow cells will require preparations of the very highest technical standard.

SOME TASKS FOR THE FUTURE

One of the obvious requirements for the future is an accepted nomenclature for the individual human somatic chromosomes, and since there is already such good general agreement regarding the standard karyotype this should not take too long to achieve. Agreement, when it is reached, should aid, and perhaps stimulate, comparison between the chromosome sets of different ethnic groups. Since there is evidently no ban to the fertility of the offspring of mixed marriages, major changes which would be detectable in the somatic chromosomes, such as unequal reciprocal translocations and pericentric inversions, are not to be expected—except perhaps as isolated and sporadic occurrences that would be quickly eliminated in view of the consequent diminished fertility. But it is not inconceivable that some ethnic groups may be differentiated by duplication—deficiency changes involving heterochromatic regions, particularly of the X and Y

chromosomes, with an associated observable difference in overall chromosome length or arm ratio. In this connection the evidence of structural polymorphism of one of the chromosomes found by Rothfels and Siminowitch (1958) in cultures of Rhesus monkey tissue should be recalled.

The study of the chromosomes in human intersexual conditions has made a good beginning and it is probable that the main outlines are already complete. However, there is much detail to be filled in and the possible role of mosaicism in contributing to their rather bewildering variations of expression remains to be evaluated. Observations on the chromosomes of other types of congenital abnormalities should continue also, and undoubtedly will. In a matter like this it is quite impossible to peer into the future and foresee what it holds.

A quite different approach and one that has not received the attention it deserves is the study of meiotic chromosomes in testicular preparations. As I have already said, these could be of particular value in assessing the frequency of non-disjunction, particularly of the sex chromosomes and the small autosomes, with its obvious relevance to the sex anomalies and mongolism. Another aspect of such studies, of course, is the possibility of identifying a chromosomal basis for cases of infertility. Reciprocal translocations and both paracentric and pericentric inversions would be expected to reduce fertility through the production of gametes with unbalanced chromosome sets. It is well known that reciprocal translocations in the mouse reduce litter size to about 50%, the precise level being a property of the individual translocation (Carter et al. 1955). No undoubted instance of inversion has been recorded in experimental mammals but on the basis of the evidence in plants a varied diminution of fertility would be expected depending upon the length of the inverted segment and the frequency of chiasma formation within it (Darlington 1937). In human matings where one partner was heterozygous for a translocation or an inversion an erratic sequence of abortions and live births would be expected. From time to time I have made enquiries as to whether such instances were known, but without success. If the male were the heterozygous partner there would be no reason to suspect a lowered sperm count or altered sperm morphology. Snell was able to demonstrate many years ago in the mouse that chromosomally unbalanced gametes could be functionally efficient. His method was to set up a mating between two appropriately marked translocation heterozygotes, and then to recover exceptional progeny that could only have developed from chromosomally balanced zygotes arising from the fertilization of unbalanced ova by complementary unbalanced sperm (Snell 1946).

The maps of certain pachytene chromosomes that have been prepared by Schultz and St. Lawrence (1949) and Yerganian (1957) also require mention. Further development of their methods and extension of the work to include dominant congenital abnormalities would be welcome and might reveal deficiencies that would not be detectable in the somatic chromosomes. But it would have to be a labour of love and there would be no sure reward. All work on meiosis in the seminiferous tubules however suffers from the very severe disadvantage that

biopsy specimens can only be taken where there are independent clinical or surgical indications.

The study of human cytogenetics has now attained the stage that the Drosophilists had reached about 40 years ago. We can hardly expect an advance as rapid and dramatic as they achieved in the twenties and thirties, particularly after the rediscovery of the salivary gland chromosomes. Nevertheless there is now a great interest on the part of the medical profession and we can look forward to the screening of large numbers of individuals, at least in Western Europe and North America, and to a steady increase in our understanding of the relationship of chromosomal abnormalities to congenital disease, to neoplasia, and to general human biology.

REFERENCES

Armstrong, C. N. 1955. Diversities of sex. *Brit. M. J.* i: 1173–11177.

Armstrong, C. N., Gray, J. E., Race, R. R. and Thompson, R. B. 1957. A case of true hermaphroditism. *Brit. M. J.* ii: 605–606.

Barr, M. L. and Bertram, E. G. 1949. A morphological distinction between neurones of the male and female, and the behavior of the nucleolar satellite during accelerated nucleoprotein synthesis. *Nature* 163: 676–677.

Bender, M. A. 1957. X-ray induced chromosome aberrations in normal diploid human tissue cultures. *Science* 126: 974–975.

Bradbury, J. T., Bunge, R. G. and Boccabella, R. A. 1956. Chromatin test in Klinefelter's syndrome. *J. Clin. Endocr.* 16: 689.

Braden, A. W. H. 1958. Influence of time of mating on the segregation ratio of alleles at the *T*-locus in the house mouse. *Nature* 181: 786–787.

Carter, T. C., Lyon, M. and Phillips, R. 1955. Gene tagged chromosome translocations in eleven stocks of mice. *J. Genet.* 53: 154–166.

Chu, E. H. Y. and Giles, N. H. 1959. Human chromosome complements in normal somatic cells in culture. *Am. J. Human Genetic.* 11: 63–79.

Danon, M. and Sachs, L. 1957. Sex chromosomes and human sexual development. *Lancet* ii: 20–25.

Darlington, C. D. 1937. *Recent Advances in Cytology*. 2nd Ed. London: Churchill.

Decourt, L., Sasso, W., Chiorboli, E. and Fernandes, J. M. 1954. Sobre o sexo genetico nas parientes com sindrome de Turner. *Rev. Ass. med. Brazil* 1: 203.

Fankhauser, G. and Humphrey, R. R. 1950. Chromosome number and development of progeny of triploid axolotl females mated with diploid males. *J. Exp. Zool.* 115: 207–240.

Fankhauser, G. and Humphrey, R. R. 1954. Chromosome number and development of progeny of triploid axolotl males crossed with diploid females. *J. Exp. Zool.* 126: 33–58.

Ford, C. E. and Hamerton, J. L. 1956. The chromosomes of man. *Nature* 178: 1020–1023.

Ford, C. E., Mole, R. H. and Hamerton, J. L. 1958. Chromosome changes in primary and transplanted reticular neoplasms of the mouse. *J. Cell. and Comp. Physiol.* 52 Suppl. 1: 235–269.

Ford, C. E., Jacobs, P. A. and Lajtha, L. G. 1958. Human somatic chromosomes. *Nature* 181: 1565–1568.

Ford, C. E., Jones, K. W., Miller, O. J., Mittwoch, U., Penrose, L. S, Ridler, M. and Shapiro, A. 1959. The chromosomes in a patient showing both mongolism and the Klinefelter syndrome. *Lancet* i: 711:713.

Ford, C. E., Micklem, H. S. and Gray, S. M. 1959. Evidence of selected proliferation of reticular cell-clones in heavily irradiated mice. *Brit. J. Radiol.* 32: 280.

Ford, C. E. and Mole, R. H. 1959. Chromosomes and carcinogenesis: Observations on radiation-induced leukaemias. *Progress in Nuclear Energy* Vol. 2. London: Pergamon Press.

FORD, C. E., POLANI, P. E., BRIGGS, J. H. AND BISHOP, P. M. F. 1959. A presumptive human XXY/XX mosaic. *Nature* **183**: 1030–1032.

FRACCARO, M., KAIJSER, K. AND LINDSTEN, J. 1959. Chromosome complement in gonadal dysgenesis. *Lancet* i: 886.

GRUMBACH, M. M. AND BARR, M. L. 1958. Cytological tests of chromosomal sex in relation to sexual anomalies in man. *Recent Progr. Hormone Res.* **14**: 255–334.

HARNDEN, D. G. A human skin culture technique used for cytological examination of different pathological conditions. *Brit. J. Exp. Path.* (in press.)

HARNDEN, D. G., BRIGGS, J. H. AND STEWART, J. S. S. 1959. Nuclear chromatin of anencephalic foetuses. *Lancet* ii: 126–127.

HSU, T. C., POMERAT, C. M. AND MOORHEAD, P. S. 1957. Heteroploid transformation in the human cell strain Mayes. *J. Nat. Cancer Inst.* **19**: 867–873.

HUNEGRFORD, D. A. 1959. Chromosomes and intersexuality. *Lancet* ii: 98.

INGVAR EK, J. AND JENSEN, C. C. 1959. Aetiology of mongolism. *Lancet* i: 1206–1207.

JACOBS, P. A., BAIKIE, A. G., COURT-BROWN, W. M., MACGREGOR, D. N., MACLEAN, M. AND HARNDEN, D. G. 1959. Evidence for the existence of a human "super female". *Lancet* ii: 423–425.

JACOBS, P. A., BAIKIE, A. G., COURT-BROWN, W. M. AND STRONG, J. A. 1959. The somatic chromosomes in mongolism. *Lancet* i: 710.

JACOBS, P. A. AND STRONG, J. A. 1959. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature* **183**: 302–303.

KODANI, M. 1957. Three diploid chromosome numbers of man. *Proc. Nat. Acad. Sc.* **43**: 285–292.

KODANI, M. 1958. The supernumerary chromosome of man. *Am. J. Human Genet.* **10**: 125–140.

LEJEUNE, J., GAUTIER, M. AND TURPIN, R. 1959a. Les chromosomes humaines en culture de tissus. *C. Rend. Acad. Sc.* **248**: 602–603.

LEJEUNE,, J., GAUTIER, M. AND TURPIN, R. 1959b. Études des chromosomes somatique de neuf enfants mongolien. *C. Rend. Acad. Sc.* **248**: 1721–1722.

MAKINO,. S. AND SASAKI, M. 1959. On the chromosome number of man. *Proc. Japan Acad.* **35**: 99–104.

MATTHEY, R. 1949. *Les chromosomes des vertébrés.* Lausanne: F. Rouge.

MOORE, K. J. 1959. Sex reversal in newborn babies. *Lancet* i: 217–219.

MORGAN, T. H., BRIDGES, C. B. AND STURTEVANT, A. H. 1925. Genetics of *Drosophila melanogaster. Bibliogr. Genet.* **2**: 1.

NOWAKOWSKI, H., LENZ, W. AND PARADA, J. 1959. Diskrepenz zwischen Chromatinbefund und genetischem Geschlecht beim Klinefeltersyndrom. *Acta Endocrinologia* **30**: 296–230.

PENROSE, L. S. 1954. Mongolian idiocy (mongolism) and maternal age. *Ann. N. York Acad. Sc.* **57**: 494–502.

PETTERSON, G. AND BONNIER, G. 1937. Inherited sex-mosaic in man. *Hereditas* **23**: 49–69.

PLUNKETT, E. R. AND BARR, M. L. 1956. Testicular dysgenesis affecting the seminiferous tubules, prinicpally with chromatin positive nuclei. *Lancet* ii: 853–856.

POLANI, P. E., HUNTER, W. E. AND LENNOX, B. 1954. Chromosomal sex in Turner's syndrome with coarctation of the aorta. *Lancet* ii: 120.

POLANI, P. E., LESSOF, M. H. AND BISHOP, P. M. F. 1956. Colour blindness in "ovarian agenesis" (gonadal dysplasia). *Lancet* ii: 118–120.

PRADER, A., SCHNEIDER, J., FRANCÉS, J. M. AND ZÜBLIN, W. 1958. Frequency of the true (chromatin-positive) Klinefelter's syndrome. *Lancet* i: 968–969.

ROTHFELS, K. H. AND SIMINOWITCH, L. 1958. The chromosome complement of the rhesus monkey (Macaca mulatta) determined in kidney cells cultivated in vitro. *Chromosoma* **9**: 163–175.

SCHULTZ, J. AND ST. LAWRENCE, P. 1949. A cytological basis for a map of the nucleolar chromosome in man. *J. Hered.* **40**: 31–39.

SHARMAN, G. B. 1959. Some effects of X-rays on living cells in the testis and bone marrow of the marsupial *Potorous tridactylus*. *Internat. J. Rad. Biol.* 1: 115–130.

SNELL, G. D. 1946. An analysis of translocations in the mouse. *Genetics* 31: 157–180.

STERN, C. 1959a. The chromosomes of man. *Am. J. Human Genet.* 11: 301–314.

STERN, C. 1959b. Colour blindness in Klinefelter's syndrome. *Nature* 183: 1452.

STEWART, J. S. S. 1959. The chromosomes in man. *Lancet* i: 833.

TAILLARD, W. AND PRADER, A. 1957. Étude génétique du syndrome de feminisation testiculaire totale et partielle. *J. Génét. Hum.* 6: 13–32.

TJIO, J. H. AND LEVAN, A. 1956. The chromosome number of man. *Hereditas* 42: 1–6.

TJIO, J. H. AND PUCK, T. T. 1958. The somatic chromosomes of man. *Proc. Nat. Acad. Sc.* 44: 1229–1237.

TJIO, J. H., PUCK, T. T. AND ROBINSON, A. 1959. The somatic chromosomal constitution of some human subjects with genetic defects. *Proc. Nat. Acad. Sc.* 45: 1008–1016.

WELSHONS, W. S. AND RUSSELL, L. B. 1959. The Y-chromosome as the bearer of male-determining factors in the mouse. *Proc. Nat. Acad. Sc.* 45: 560–566.

WESTERGAARD, M. 1940. Studies on cytology and sex determination in polyploid forms of *Melandrium album*. *Dansk botan. Arkiv.* 10: 1–131.

WILKINS, L., GRUMBACH, M. M. AND VAN WYK, J. J. 1954. Chromosomal sex in ovarian agenesis. *J. Clin. Endocr.* 14: 1270.

YERGANIAN, G. 1957. Cytological maps of some isolated human pachytene chromosomes. *Am. J. Human Genet.* 9: 42–54.

Note added in proof. The value of reviews such as this is largely bibliographical, and since the subject is developing so rapidly references are given below to a number of publications that were accidentally omitted from the main bibliography or appeared after the manuscript was submitted. Among them is a full report by Hungerford et al. on the true hermaphrodite discussed in page 110. The authors consider mosaicism to be unlikely, but on the evidence presented, XX/XXY mosaicism involving gonads but not bone marrow cannot yet be rigorously excluded.

BAIKIE, A. G., COURT BROWN, W. M., JACOBS, P. A. AND MILNE, J. S. 1959. Chromosome studies in human leukaemia. *Lancet* ii: 425–428.

BÖÖK, J. A., FRACCARO, M. AND LINDSTEN, J. 1959. Cytogenetical observations in mongolism. *Acta paediat.* 48: 453–468.

FORD, C. E., JONES, K. W., POLNIA, P. E., DE ALMEIDA, J. C. AND BRIGGS, J. H. 1959. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet* i: 711–713.

HARNDEN, D. G. AND ARMSTRONG, C. N. 1959. Chromosomes of a true hermaphrodite. *Brit. M. J.* ii: 1287–1288.

HARNDEN, D. G. AND STEWART, J. S. S. 1959. Chromosomes in a case of pure gonadal dysgenesis. *Brit. M. J.* ii: 1285–1287

HUNGERFORD, D. A., DONNELLY, A. J., NOWELL, P. C. AND BECK, S. 1959. The chromosome constitution of a human phenotypic intersex. *Am. J. Human Genet.* 11: 215–236.

JACOBS, P., BAIKIE, A. G., COURT BROWN, W. M., FORREST, H., ROY, J. R., STEWART, J. S. S. AND LENNOX, B. 1959. Chromosomal sex in the syndrome of testicular feminisation. *Lancet* ii: 591–592.

NILSSON, I. M., BERGMAN, S., REITALU, J. AND WALDENSTRÖM, J. 1959. Haemophilia A in a "girl" with male sex-chromatin pattern. *Lancet* ii: 264–266.

TURPIN, R., LEJEUNE, J., LAFOURCADE, J. AND GAUTIER, M. 1959. Aberrations chromosomiques et maladies humaines. La polydysspondylie a 45 chromosomes. *C. rend. Acad. Sc.*, 248: 3636–3638.

Sexual Dimorphism in Interphase Nuclei[1]

MURRAY L. BARR

Department of Microscopic Anatomy, University of Western Ontario, London, Canada

THE SUBJECT OF SEXUAL DIMORPHISM in interphase nuclei has only a tenuous relationship with genetics, since the presence or absence of sex chromatin yields no information concerning the autosomes and, in certain sex anomalies, may give incomplete information as to the nature of the sex chromosome complex. Such interest as the subject may have in this symposium arises from three sources. Nuclear sexual dimorphism is the basis for relatively simple tests that are useful diagnostic aids when dealing with errors of sex development in man; the tests have drawn attention to the possibility of unusual sex chromosome complexes in two syndromes; and the basis of nuclear sexual dimorphism, in terms of chromosome morphology and behaviour, is a challenging problem to the cytologist and cytogeneticist.

THE SEX CHROMATIN

There are recent accounts of the structure of interphase nuclei according to sex (Grumbach and Barr, 1958; Barr, 1959), and only the main observations are recalled at this time. The imprint of sex is found in a special chromocenter, the sex chromatin, that is distinctive of the female in certain mammals. Like chromocenters generally, the sex chromatin is basophilic and Feulgen-positive. But it is larger than the other chromatin particles and is usually located against the inner surface of the nuclear membrane (Fig. 1, a & b). The sex chromatin is studied to best advantage in the large, vesicular nuclei of neurones, although in some animals (e.g., cat) it lies against the large nucleolus of this particular type of cell. Sexual dimorphism of the nuclei is present from early stages of embryonic development and has been described in cells cultured *in vitro*.

A characteristic chromatin pattern for male and female occurs in most types of cells in man, monkey and carnivores. In the order Artiodactyla, which is comprised of some of the hoofed animals, the sex chromatin is visible in neuronal nuclei of females but not of males, while the nuclear chromatin is too coarse in most other types of cells for satisfactory demonstration of this detail. With the exception of a few cell types, the nuclei of rodents and the rabbit (a lagomorph) contain numerous large masses of chromatin and are not well suited to the study

[1] Part of a Symposium on Cytology in Human Genetics held on Sept. 2, 1959, under the joint sponsorship of the American Society of Human Genetics and the Genetics Society of America during the meetings of the American Institute of Biological Sciences at Pennsylvania State University, August 30 to Sept. 3, 1959.

Studies on which this report is based were supported by the National Research Council and National Health Grants of Canada, the J. P. Bickell Foundation and the D. H. McDermid Medical Research Fund.

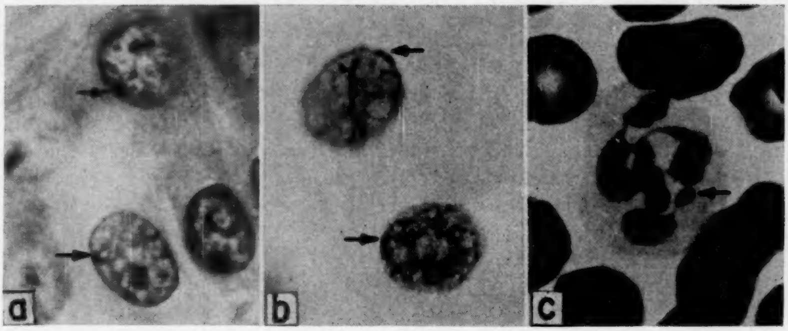

FIG. 1. a. Nuclei in the stratum spinosum of a skin biopsy specimen from a normal female. Hematoxylin and eosin stain. b. Nuclei in an oral smear preparation from a normal female. Thionin stain. c. Neutrophil leucocyte in a blood film from a normal female. Giemsa stain. 1800 ×.

of sexual dimorphism. Nuclei of the Virginia oppossum are unusual in that a definite chromocenter is present in the nuclei of both sexes, but it is significantly larger in females than in males.

DERIVATION OF THE FEMALE-SPECIFIC CHROMOCENTER

The fact of nuclear sexual dimorphism has been firmly established by many independent studies, but the derivation of the sex chromatin remains an unsolved problem. The following considerations bear on this important and vexatious question.

The sex chromatin, if it has a basis in common with other chromocenters, is probably derived from heterochromatic regions of chromosomes. The concept of a chromosomal origin is supported by the finding of two or more masses of sex chromatin in nuclei that are known to be hyperdiploid. Examples are malignant cells (Moore and Barr, 1954 a) and cells of amniotic epithelium (Klinger and Schwarzacher, 1958). Chromosomes that bear sex-determining genes are likely to be involved and the sex chromosomes are the obvious candidates, since they alone are distinctly different in the two sexes.

An alternative hypothesis was put forward by Segal and Nelson (1957), who suggested that the sex chromatin may be derived from a pair of autosomes that bears male-determiners. It was postulated that these autosomes, or regions of them, might be positively heteropycnotic and genetically inert in females, but isopycnotic and genetically active in males. This intriguing hypothesis is based on the genetic balance theory of sex determination, which states that the autosomes carry the male-determiners and implies that the Y chromosome has a passive role in sex determination. But this theory must be reassessed for mammals in the light of recent evidence that the Y chromosome bears male-determining genes in the mouse (Russell, Russell and Gower, 1959; Welshons and Russell, 1959) and in man (Jacobs and Strong, 1959; Ford *et al.*, 1959 b).

Reverting to a possible relationship between sex chromatin and sex chromosomes, Graham and Barr (1952) and Moore and Barr (1953) suggested that the sex chromatin may represent heterochromatic regions of homologous X chromosomes in somatic pairing. It was implied that the property of heterochromaticity was less well developed in the XY complex of somatic cells, compared with the XX complex, or that the XY complex failed to form a conspicuous chromocenter because of the small size of the Y chromosome. This suggestion found some support in the bipartite appearance of the sex chromatin (Klinger, 1958) and from other detailed studies of interphase nuclei. For example, Reitalu (1957) described details in hepatic cell nuclei of human embryos that were compatible with somatic pairing of the X chromosomes. The heterochromatic portions of these chromosomes seemed to form the bipartite sex chromatin, while the euchromatic portions were visible as delicate threads attached to the nucleolus. In cells of male embryos, the single X chromosome formed a small chromocenter but identification of the Y chromosome was difficult. These structures were duplicated in tetraploid nuclei of both sexes. Reitalu also noted that the two X chromosomes present in tetraploid nuclei of males were not associated in somatic pairing. This observation may be related to the finding of Moore and Barr (1954 a) that the frequency of nuclei with a sex chromatin-like mass conformed to normal values in malignant tumours in males, while nuclei with two, or even three, masses of sex chromatin occurred in malignant tumours in females. Reitalu's observations were confirmed on several points by Serr *et al.* (1958), who studied human thyroid epithelial cells cultured *in vitro*. The presence of an XXY complex in patients with the Klinefelter syndrome and female-type nuclei (Jacobs and Strong, 1959; Ford *et al.*, 1959 b) and of an XO arrangement in patients with Turner's syndrome and male-type nuclei (Ford *et al.*, 1959 a; Tjio, Puck and Robinson, 1959) is compatible with the view that the sex chromatin is an XX chromosome derivative, but does not constitute proof that this is so.

Perhaps the principal weakness of the foregoing hypothesis is the necessity of invoking somatic pairing of the X chromosomes. While pairing of homologous chromosomes in somatic cells is well known in many insects and has been described for the newt and frog (Boss, 1955), there seems to be little evidence of a conclusive nature that bears on this matter so far as mammals are concerned. For example, somatic association of the X chromosomes has been recorded in epithelial cells of ovarian follicles and mammary gland in the mouse, but could not be detected in other types of cells that were examined (Ohno, Kaplan and Kinosita, 1959a; Ohno, Kovacs and Kinosita, 1959). Recent studies suggest a possible way out of the impasse that one would not have predicted. Ohno, Kaplan and Kinosita (1959b) made the surprising observation that only one of the X chromosomes of female rat liver cells was positively heteropycnotic, while both X and Y chromosomes in similar cells of males were isopycnotic with respect to the autosomes. The positively heteropycnotic X chromosome, which may be of paternal origin, appeared to be folded back on itself in early prophase. Ohno and his collaborators point out that the sex chromatin may be derived from a

single X chromosome and that the configuration of this chromosome may explain the bipartite appearance of the sex chromatin.

The observations of Kosin and Ishizaki (1959) on nuclei of the domestic chicken are consistent with a derivation of the sex chromatin from a single chromosome. They found the characteristic sexual dimorphism that has been described for mammals, the sex chromatin being present in the female. Since the female of the domestic chicken is said to possess a ZO sex chromosome constitution (Miller, 1938; Newcomer, 1957; Witschi, 1959), it follows that the sex chromatin, if related to the sex chromosomes at all, is a derivative of the single Z chromosome of the female in this species.

Extension of these recent observations to those mammals whose nuclei have a well defined sexual dimorphism, and particularly to man, will be of the greatest interest in interpretation of chromatin pattern and sex chromosome consititution of patients with the syndromes of Turner and Klinefelter. It is gratifying to find cytologists with experience in the study of chromosomes taking an interest in this problem.

APPLICATION OF NUCLEAR SEXUAL DIMORPHISM TO HUMAN SEX ANOMALIES

(a) *Tests in current use*

The *skin biopsy method* was the first to be introduced (Moore, Graham and Barr, 1953), and it has been largely replaced by methods that avoid any surgical procedure. Various staining methods can be used (Moore and Barr, 1954 b; Klinger and Ludwig, 1957), but the preparations must be of high technical quality. Cells in the stratum spinosum and hair follicles are the most satisfactory. Sex chromatin can be identified in from 60 to 80 per cent of their nuclei in normal females (Fig. 1 a), while in normal males a rather smaller chromocenter, of unknown significance, is encountered in less than 10 per cent of nuclei. The procedures used for preparing sections of skin for this purpose are applicable to other tissues that may be obtained at operation or post mortem.

The *oral smear method* is used extensively, mainly because of its simplicity (Moore and Barr, 1955; Marberger, Boccabella and Nelson, 1955). A variety of staining methods may be used, provided that other technical requirements are satisfied. The method of Klinger and Ludwig (1957) has the advantage of eliminating the staining of bacteria. This method includes hydrolysis of the preparation in N HCl at 56°C for *not more* than 5 min., prior to staining with thionin. The details are recorded elsewhere (Barr, in press). Some prefer to stain the smears with cresyl echt violet or another basic dye, without prior treatment other than the usual fixation.

Smear preparations of the oral mucosa are so easy to obtain that they are likely to be studied from large numbers of persons. So a word of caution may be advisable. In general, the nuclei in oral smears display the same sexual dimorphism that is found in nuclei of tissues generally (Fig. 1 b). But the tendency of the female sex chromosome complex (or part of it) to form a distinctive chromocenter is not as pronounced in nuclei of oral epithelium as it is in nuclei at other sites.

Consequently, the proportion of healthy-appearing nuclei that contain recognizable sex chromatin may be as low as 25 or 30 per cent in oral smears from normal females, although higher figures, even up to 80 per cent, are often obtained. Further, the sex chromatin is smaller than usual in some preparations from normal females and may be greatly flattened against the nuclear membrane (Fig. 1 b, upper nucleus). On the credit side, a chromocenter that could be confused with the sex chromatin is very rarely present in oral smear preparations from normal males.

In some preparations from both sexes, many nuclei contain a spherical chromocenter that lies well away from the nuclear membrane. The position of this chromocenter is such that it should cause no difficulty in the interpretation of the preparation. As an unusual occurrence, satisfactory oral smears cannot be obtained, probably because of an unhealthy condition of the oral mucosa, and one of the other methods must then be used. The oral smear test has proved reliable, in our hands, in a study of about 3000 individuals. Anyone who proposes to apply this method to problems of sexual differentiation should first acquire technical and observational experience from the study of a series of preparations from normal males and females.

The sex chromatin is more prominent in nuclei of vaginal smears than it is in those of oral smears (Carpentier, Stolte and Visschers, 1956; Guard, 1959). Vaginal smears could be obtained from patients with suspected gonadal dysgenesis (Turner's syndrome) or testicular feminization.

The *neutrophil test*, which is based on a type of sexual dimorphism peculiar to polymorphonuclear leucocytes, was discovered by Davidson and Smith (1954) and has received abundant confirmation (e.g., Kosenow and Scupin, 1956; Briggs, 1958; Wiedemann, Tolksdorf and Romatowski, 1958). In normal females, a small proportion of neutrophils (1 to 10 per cent, average about 3 per cent) have an accessory nuclear lobule with a filamentous attachment to a lobe of the nucleus (Fig. 1 c). The accessory lobule has average dimensions of $1.2\,\mu \times 1.6\,\mu$ and it occurs more frequently with increasing nuclear lobulation. This configuration occurs with the greatest rarity, if at all, in neutrophils of normal males. The relation of the female-specific nuclear lobule to the sex chromatin is not known, but results of the oral smear and neutrophil tests have the same significance as diagnostic aids in the sex anomalies.

In normal males, there are frequently nuclear projections that, while they are smaller, have the same shape as the accessory lobule of females. Occasionally, "the small clubs" of the male are sufficiently large as to make interpretation difficult, unless one has had considerable experience in studying the many variants of nuclear morphology in neutrophil leucocytes.

(b) *Application of the tests to sex anomalies in man*

The foregoing cytological tests have a diagnostic value and a theoretical bearing on errors of sex development.

(i) *female pseudohermaphroditism.* In most subjects with female gonads and internal genitalia, but with intersexual external genitalia, the anomaly is caused

by fetal adrenocortical hyperplasia and excessive elaboration of androgenic steroids during the third trimester (the adrenogenital syndrome in females). A similar abnormality may result from the administration of progestins to the mother during pregnancy (Wilkins and Jones, 1958). The genetic sex determining mechanism is not involved in either instance and the nuclei always have a female chromatin pattern, as expected. The external genitalia are not necessarily distinctive of female pseudohermaphroditism, as compared with male pseudohermaphroditism, and the main value of the tests is as an aid in distinguishing between the two conditions.

(ii) *male pseudohermaphroditism.* Subjects with male gonads and an intersexual development of the remainder of the reproductive system appear to arise through a defective evocator action on the part of the embryonal testes (Jost, 1953). Although family studies suggest that there is a genetic etiological factor, there is no particular reason for suspecting an unusual sex chromosome complex. In any event, the nuclear chromatin pattern is always male.

(iii) *testicular feminization.* This special form of male pseudohermaphroditism is characterized by normally female external genitalia and good development of female secondary sex characteristics at puberty. The anomaly follows a maternal inheritance pattern and is probably based on a defect at the gene level. The nuclear chromatin pattern is consistently male.

(iv) *true hermaphroditism.* Subjects with both ovarian and testicular tissue and intersexual development of the reproductive tract may have either a female or a male chromatin pattern, more frequently the former. Hungerford *et al.* (1959) reported the presence of an XX complex in a true hermaphrodite whose nuclei contained sex chromatin. But chromosome studies need to be done on additional patients to explore the possibility of an abnormal sex chromosome complex, such as XXY in some true hermaphrodites with female-type interphase nuclei.

(v) *gonadal dysgenesis* (*Turner's syndrome*) (Fig. 2). In patients with gonadal dysgenesis (called Turner's syndrome when there are certain added congenital defects), the "gonads" consist of slender streaks of connective tissue. The rest of the reproductive system has a female morphology. Shortness of stature is almost the rule and other anomalies, of the skeletal and cardiovascular systems especially, occur in a proportion of subjects. Urinary excretion of pituitary gonadotrophins is elevated after the age of 10 years and secondary sex characteristics fail to develop at puberty.

The nuclei have a male chromatin pattern in 80 per cent of patients with the syndrome of gonadal dysgenesis (Grumbach, Van Wyk and Wilkins, 1955; Grumbach and Barr, 1958). It has now been shown that the male chromatin pattern is correlated with the presence of an XO sex chromosome complex or, in some patients, with an XX/XO mosaicism (Ford *et al.*, 1959 a; Tjio, Puck and Robinson, 1959; Ford, this Symposium; Fraccaro, this Symposium). It appears, therefore, that XO and XY complexes are not equivalent in the genetic sex determining mechanism of man, but rather that the Y chromosome is an important carrier of male-determiners. The XO complex is incomplete for either

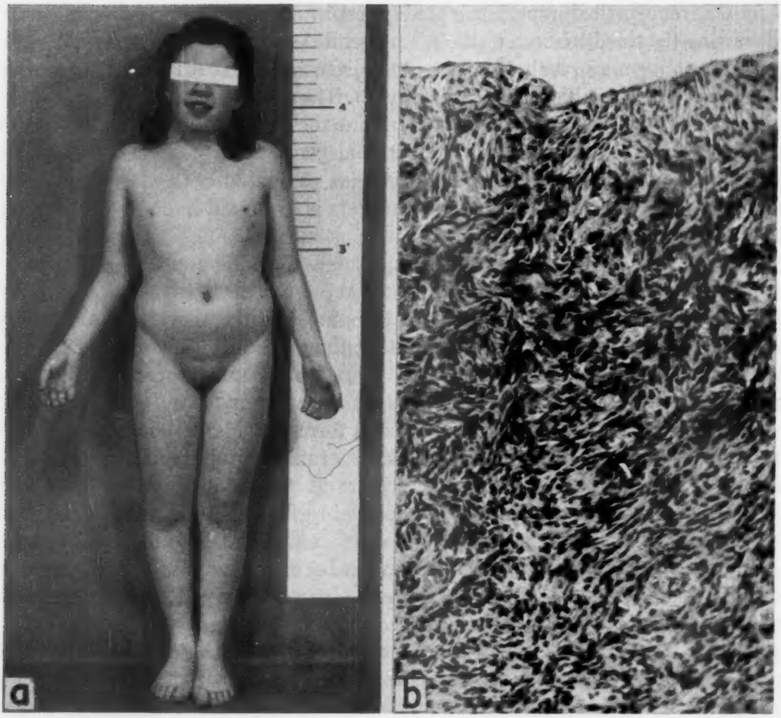

FIG. 2. a. 16-year-old patient with syndrome of gonadal dysgenesis (Turner's syndrome) and a male chromatin pattern. Courtesy of Dr. J. C. Rathbun, Professor of Pediatrics, University of Western Ontario. b. Histological structure of typical "streak gonad" in the syndrome of gonadal dysgenesis. Hematoxylin and eosin stain. 280 ×. Courtesy of Dr. M. M. Grumbach, Columbia-Presbyterian Medical Center.

sex and definitive gonads fail to develop from the undifferentiated embryonal gonads. The development of a female reproductive tract is probably the result of an inherent tendency of all embryos to feminize in the absence of a masculinizing inductor of testicular origin (Jost, 1953).

(vi) *seminiferous tubule dysgenesis* (*Klinefelter's syndrome*) (Fig. 3). Phenotypic males with the syndrome of seminiferous tubule dysgenesis or Klinefelter's syndrome have small testes with varying degrees of fibrosis and hyalinization of the seminiferous tubules. Spermatogenesis is scanty if it occurs at all. The legs are usually long in relation to the length of the trunk and eunuchoid traits, such as poor growth of facial hair, are frequently seen. Urinary excretion of pituitary gonadotrophins is usually elevated after puberty.

The nuclei have a female chromatin pattern in about three-fourths of subjects who qualify on clinical grounds for inclusion in the Klinefelter syndrome (Nelson, 1957; Raboch, 1957). The finding of female-type nuclei in these phe-

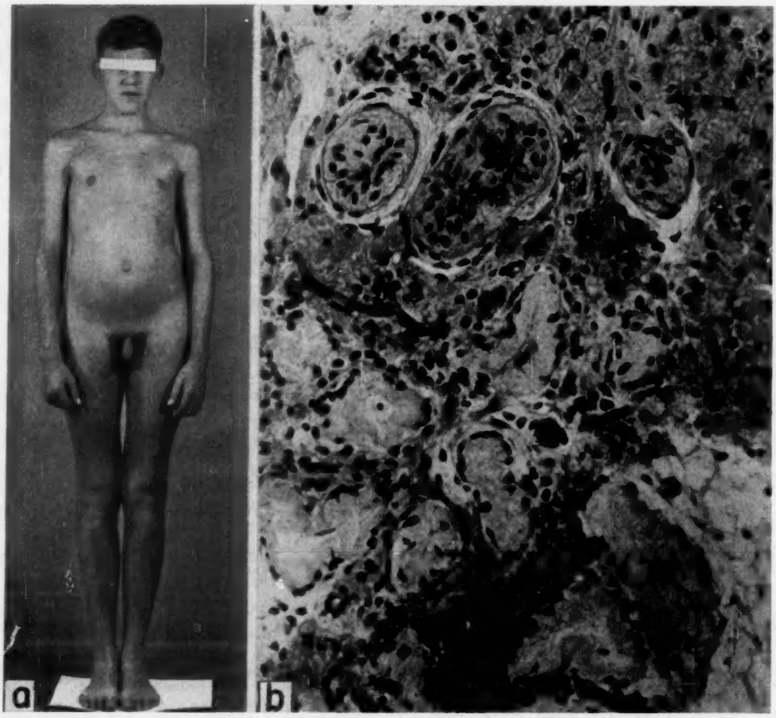

FIG. 3. a. 15-year-old patient with seminiferous tubule dysgenesis (Klinefelter's syndrome) and a female chromatin pattern. b. Testicular histopathology in Klinefelter's syndrome. Some of the tubules are entirely hyalinized, others contain only Sertoli cells. The Leydig cells are in large aggregates. Hematoxylin and eosin stain. 125 ×.

notypic males is related to the presence of an XXY sex chromosome complex or an XX/XXY mosaicism (Jacobs and Strong, 1959; Ford *et al.*, 1959b). The gonads have a nearly normal testicular structure before puberty (Siebenmann and Prader, 1958; Ferguson-Smith, 1959). It appears, therefore, that the male-determining factors carried by the Y chromosome, together with such male-determiners as there may be on autosomes, almost entirely override the female-determiners on the two X chromosomes. But there is an abnormal response of the seminiferous tubules to the hormonal changes of puberty and they rapidly undergo regressive changes.

SUMMARY

The presence of a distinctive chromocenter, the sex chromatin, in interphase nuclei is a female characteristic in man and some other mammals. This nuclear detail provides a useful diagnostic aid in several errors of sex development in man. Absence of sex chromatin has been correlated with an XO complex or an

XX/XO mosaicism in the syndrome of gonadal dysgenesis. Presence of sex chromatin has been correlated with an XXY complex or an XX/XXY mosaicism in the syndrome of seminiferous tubule dysgenesis.

Critical and analytical work by students of chromosome morphology and behaviour is needed to establish the basis for the sexual dimorphism of interphase nuclei.

REFERENCES

BARR, M. L. 1959. Sex chromatin and phenotype in man. *Science* 130: 679–685.

BARR, M. L. Cytological tests of chromosomal sex. *Recent Advances Clin. Path.* (Churchill, London), vol. 3. (in press)

BOSS, J. M. N. 1955. The pairing of somatic chromosomes: a survey. *Texas Rep. Biol. M.* 13: 213–221.

BRIGGS, D. K. 1958. The individuality of nuclear chromatin with particular reference to polymorphonuclear neutrophil leucocytes. *Blood* 13: 986–1000.

CARPENTIER, P. J., STOLTE, L. A. M., AND VISSCHERS, G. P. 1956. Determination of genetic sex by the vaginal smear. *J. Clin. Endocr. Metab.* 16: 155–160.

DAVIDSON, W. M., AND SMITH, D. R. 1954. A morphological sex difference in the polymorphonuclear neutrophil leucocytes. *Brit. M. J.* ii: 6–7.

FERGUSON-SMITH, M. A. 1959. The prepubertal testicular lesion in chromatin-positive Klinefelter's syndrome (primary micro-orchidism) as seen in mentally handicapped children. *Lancet* i: 219–222.

FORD, C. E., JONES, K. W., POLANI, P. E., DE ALMEIDA, J. C., AND BRIGGS, J. H. 1959 a. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet* i: 711–713.

FORD, C. E., POLANI, P. E., BRIGGS, J. H., AND BISHOP, P. M. F. 1959 b. A presumptive human XXY/XX mosaic. *Nature* 183: 1030–1032.

GRAHAM, M. A., AND BARR, M. L. 1952. A sex difference in the morphology of metabolic nuclei in somatic cells of the cat. *Anat. Rec.* 112: 709–723.

GRUMBACH, M. M., AND BARR, M. L. 1958. Cytologic tests of chromosomal sex in relation to sexual anomalies in man. *Recent Progr. Hormone Res.* 14: 255–324.

GRUMBACH, M. M., VAN WYK, J. J., AND WILKINS, L. 1955. Chromosomal sex in gonadal dysgenesis (ovarian agenesis): relationship to male pseudohermaphrodism and theories of human sex differentiation. *J. Clin. Endocr. Metab.* 15: 1161–1193.

GUARD, H. R. 1959. A new technic for differential staining of the sex chromatin, and the determination of its incidence in exfoliated vaginal epithelial cells. *Am. J. Clin. Path.* 32: 145–151.

HUNGERFORD, D. A., DONNELLY, A. J., NOWELL, P. C., AND BECK, S. 1959. The chromosome constitution of a human phenotypic intersex. *Am. J. Human Genet.* 11: 215–236.

JACOBS, P. A., AND STRONG, J. A. 1959. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature* 183: 302–303.

JOST, A. 1953. Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Progr. Hormone Res.* 8: 379–413.

KLINGER, H. P. 1958. The fine structure of the sex chromatin body. *Exp. Cell. Res.* 14: 207–211.

KLINGER, H. P., AND LUDWIG, K. S. 1957. A universal stain for the sex chromatin body. *Stain Techn.* 32: 235–244.

KLINGER, H. P., AND SCHWARZACHER, H. G. 1958. Sex chromatin in polyploid nuclei of human amnion epithelium. *Nature* 181: 1150–1152.

KOSENOW, W., AND SCUPIN, R. 1956. Die Bestimmung des Geschlechts mit Hilfe einer Kernanhangsformel der Leukocyten. *Acta haemat.* 15: 349–363.

KOSIN, I. L., AND ISHIZAKI, H. 1959. Incidence of sex chromatin in Gallus domesticus. *Science* 130: 43–44.

Marberger, E., Boccabella, R. A., and Nelson, W. O. 1955. Oral smear as a method of chromosomal sex detection. *Proc. Soc. Exp. Biol.* **89**: 488–489.

Miller, R. A. 1938. Spermatogenesis in a sex-reversed female and in normal males of the domestic fowl, *Gallus domesticus*. *Anat. Rec.* **70**: 155–189.

Moore, K. L., and Barr, M. L. 1953. Morphology of the nerve cell nucleus in mammals, with special reference to the sex chromatin. *J. Comp. Neur.* **98**: 213–231.

Moore, K. L., and Barr, M. L. 1954 a. The sex chromatin in human malignant tissues. *Brit. J. Cancer* **11**: 384–390.

Moore, K. L., and Barr, M. L. 1954 b. Nuclear morphology, according to sex, in human tissues. *Acta anat.* **21**: 197–208.

Moore, K. L., and Barr, M. L. 1955. Smears from the oral mucosa in the detection of chromosomal sex. *Lancet* ii: 57–58.

Moore, K. L., Graham, M. A., and Barr, M. L. 1953. The detection of chromosomal sex in hermaphrodites from a skin biopsy. *Surg. Gyn. Obst.* **96**: 641–648.

Nelson, W. O. 1957. The "Klinefelter syndrome". *Fertil. & Steril.* **8**: 527–536.

Newcomer, E. H. 1957. The mitotic chromosomes of the domestic fowl. *J. Hered.* **48**: 227–234.

Ohno, S., Kaplan, W. D., and Kinosita, R. 1959a. Somatic association of the positively heteropycnotic X-chromosomes in female mice (*Mus musculus*). *Exp. Cell Res.* **15**: 616–618.

Ohno, S., Kaplan, W. D., and Kinosita, R. 1959b. Formation of the sex chromatin by a single X-chromosome in liver cells of *Rattus norvegicus*. *Exp. Cell Res.* **18**: 415–418.

Ohno, S., Kovacs, E. T., and Kinosita, R. 1959. On the X-chromosomes of mouse mammary carcinoma cells. *Exp. Cell Res.* **16**: 462–465.

Raboch, J. 1957. Thirty-one men with female sex chromatin. *J. Clin. Endocr. Metab.* **17**: 1429–1439.

Reitalu, J. 1957. Observations on the so-called sex chromatin in man. *Acta genet. med. gemell.* **6**: 393–402.

Russell, W. L., Russell, L. B., and Gower, J. S. 1959. Exceptional inheritance of a sex-linked gene in the mouse explained on the basis that the X/O sex chromosome constitution is female. *Proc. Nat. Acad. Sc.* **45**: 544–560.

Segal, S. J., and Nelson, W. O. 1957. Developmental aspects of human hermaphrodism: the significance of sex chromatin patterns. *J. Clin. Endocr. Metab.* **17**: 676–692.

Serr, D. M., Ferguson-Smith, M. A., Lennox, B., and Paul, J. 1958. Representation of the X-chromosome in intermitotic nuclei in man. *Nature* **182**: 124.

Siebenmann, R., and Prader, A. 1958. Das echte Klinefelter-Syndrom vor der Pubertät. *Schweiz. med. Wschr.* **88**: 607–610.

Tjio, J. H., Puck, T. T., and Robinson, A. 1959. The somatic chromosomal constitution of some human subjects with genetic defects. *Proc. Nat. Acad. Sc.* **45**: 1008–1016.

Welshons, W. J., and Russell, L. B. 1959. The Y-chromosome as the bearer of male determining factors in the mouse. *Proc. Nat. Acad. Sc.* **45**: 560–566.

Wiedemann, H. R., Tolksdorf, M., and Romatowski, H. 1958. Über das Kerngeschlecht der weissen Blutzellen und den Wert der Diagnose des chromosomalen Geschlechts aus dem Blut, verglichen mit anderen Methoden. *Med. Mschr.* **12**: 665–668.

Wilkins, L., and Jones, H. W. 1958. Masculinization of the female fetus. *Obst. Gyn.* **11**: 355.

Witschi, E. 1959. Age of sex-determining mechanisms in vertebrates. *Science* **130**: 372–375.

Some Systems for the Genetic Analysis of Mammalian Cells[1]

LEO SACHS AND MATHILDE KRIM

Department of Experimental Biology, Wiezmann Institute of Science, Rehovoth, Israel

WHEN STUDYING the possibilities for a cellular analysis in the field of human genetics, one has to consider two questions: A. What are the cytological and gegetic markers that can be used? and B. What are the experimental systems that can be developed? In this framework, and using illustrations from mice where necessary, we would like to consider some of the information on the following: 1. Chromosome variation. 2. Antigenic variation. 3. Stable cell-virus associations.

CHROMOSOMAL VARIATION

The data on human chromosome variation in intersexes and mongoloids has been discussed by the three perivous speakers. The "nuclear sexing" method, discovered by Barr, had led to predictions of variation in sex chromosome constitution and chromosome mosaics (Danon and Sachs, 1957), and the existence of such variation and of mosaics has been demonstrated by somatic chromosome counts by Ford and confirmed by others (papers by Ford and Fraccaro in this symposium). We have also found two patients with Turner's syndrome with two sex chromocenters in some cells and none in others. There thus seem to be mosaics which may be either XXXX/XO or XXX/XO. These variations can serve as material for the study of genetic differences between cells that differ by one or more chromosomes. The data on chromosome variation in intersexes (Fig. 1), further show that the human Y chromosome has male-determining properties, and a similar conclusion has recently been reached for the mouse (Welshons and Russell, 1959).

ANTIGENIC VARIATION

The above conclusion on the male-determining properties of the Y chromosome makes it worth while to search for genes linked to the Y that may serve as genetic markers. Previous data on a number of characters that were supposed to show Y-linkage in man have been found capable of alternative interpretation (Stern, 1957) so that so far no other Y-linked characters have been established. At the cellular level, antigenic differences would be useful markers. In mice, skin grafts within an inbred strain can be rejected when male tissue is grafted onto females (Eichwald and Silmser, 1955) and this is a general phenomenon for all strains

[1] Part of a Symposium on Cytology in Human Genetics held on Sept. 2, 1959, under the joint sponsorship of the American Society of Human Genetics and the Genetics Society of America during the meetings of the American Institute of Biological Sciences at Pennsylvania State University, Aug. 30 to Sept. 3, 1959.

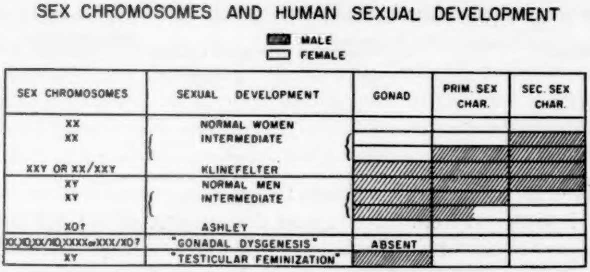

FIG. 1. Summary of results on chromosome variation in human intersexes

TABLE 1. SUCCESS OF SKIN GRAFTS WITHIN VARIOUS STRAINS OF MICE

	C57BL	A	C3H	BRS	BALB/C
♀ → ♀	+	+	+	+	NT
♀ → ♂	+	+	+	+	+
♂ → ♂	+	+	+	+	+
♂ → ♀	−	−	−	−	−

\+ = successful. NT = Not Tested
− = unsuccessful.

of mice studied (Table 1) (Sachs and Heller, 1958). One of the possible explanations of the data is that there exists a Y-linked antigenic difference. However, this rejection of male grafts by females in mice is not accompanied by any detectable circulating antibodies (Sachs and Heller, 1958), so that for *in vitro* genetic studies this character would not be the easiest to work with.

A more suitable type of antigenic marker in mice is the H-2 locus, where there exists a pseudo-allelic series with similarities to the Rh locus in man. With H-2, crossing-over has been demonstrated within the locus (Gorer, 1956). The antigens at this locus, in addition to producing the destruction of tissue grafts, elicit the production of circulating hemagglutinins that can be used for *in vitro* studies. The distribution of the hemagglutinogens in different strains of mice is shown in Table 2. These antigens, and those that elicit the production of cytotoxins (Gorer and O'Gorman, 1956), have served as good genetic markers for the analysis of antigenic changes that can occur in somatic cells.

An example of such an analysis (Sachs and Gallily, 1956; Sachs and Feldman,

TABLE 2. ANTIGENIC COMPONENTS OF THE H-2 SYSTEM (AFTER GORER)

Pseudo-allelle	Hemagglutinating Antigens
$H-2^a$	C D E F K
$H-2^b$	D^b E F
$H-2^d$	C D E^d F
$H-2^{d1}$	C D^1 E^d F
$H-2^k$	C E K
$H-2^p$	C E ? P
$H-2^q$	C E F Q
$H-2^s$	C E F S

TABLE 3. PROPERTIES OF NORMAL TISSUE AND THREE TUMORS ORIGINATING IN C3H MICE

Tissue	H-2 Hemagglutinating Antigens	Cytotoxic Antigens	Homotransplantability	Model Chromosome Number
Normal C3H	CEK	A'C'M'	−	40
Tumor 6C3HED	CEK	C'	−	40
Tumor MCIM	CEK	A'C'M'	±	~65
Tumor MCIA	CK	(A')	+	~80

1958) is given in Table 3. This shows the antigenic constitution of cells from normal C3H mice, and from three tumors that originated in C3H mice but with different degrees of homotransplantability and different modal chromosome numbers. It can be seen that these antigenic markers can be used to study mutation both at the diploid level and in cells with higher chromosome numbers. The further study of mutation with these markers, including the use of coisogenic lines of mice (Klein and Klein 1958, Mitchinson, 1956) combined with artificial mutagenesis, would seem to be a good approach towards an understanding of the genetics of somatic mammalian cells.

Another system that may be useful was found during experiments on the induction of immunological tolerance to cells from strain-specific tumors (Koprowski, Theis and Love, 1956). These experiments showed not only that there was as expected, a change in the response of the treated host, but that there was also a change in the tumor cells. As a result of growth in what may have been only a partially tolerant host, the tumor cells had acquired the property of homotransplantability, and an analysis of the hemagglutinogens and cytotoxinogens (Feldman and Sachs, 1958) showed that there had also been antigenic changes in conjunction with changes in the modal chromosome number. The results for a tumor originating in a DBA mouse, and for its subline, DBA-ICR, adapted in this manner to homotransplantability, are given in Table 4. It does not appear that these changes were due to selection from the original cell population, and it seems possible that genetic changes in the cells have been produced directly as a result of the conditions prevailing during the adaptation. An *in vitro* study of the conditions necessary for these changes would be the next step in the further analysis of this phenomenon.

Studies on cells with chromosome numbers higher than the diploid have the disadvantage in that there may be complications due to gene dosage effects, and it would of course be preferable to work with cells that can be maintained as stable diploids. These possible complications exist in all the old established tissue culture lines, none of which is diploid. The establishment of stable diploids in culture would thus be one of the first pre-requisites for genetic analysis, and it appears that the culture of such lines can now be achieved (Puck, 1958, Fraccaro, this symposium). In addition to their use for the study of antigenic changes in the mouse, such cultures could also be of value in the analysis of antigenic

TABLE 4. PROPERTIES OF SHA DBA TUMOR AND ITS ADAPTED DBA-ICR SUBLINE

Tumor	H-2 Hemagglutinating Antigens	Cytotoxic Antigens	Homotransplantability	Model Chromosome Number
DBA	CDE^dF	D'	−	42
DBA-ICR	CDE^dF?	−	+	76–78

changes in human cells. With the blood group antigens as markers, homozygotes and heterozygotes are readily available, and the use of mixed cell agglutination (Coombs, Bedford, and Rouillard, 1956, Sachs, Feldman, and Danon, 1956) provides an appropriate technic for the identification of mutants in individual cells. Human blood groups have also proved useful as markers for studies on somatic variation *in vivo* (Atwood and Scheinberg, 1958).

STABLE CELL-VIRUS ASSOCIATIONS

Given suitable markers, the genetic analysis of somatic cells further depends on the possibilities of mating (Lederberg, 1958), somatic crossing-over, and transformation, all of which seem worth while exploring. In addition, however, there is a possibility of finding stable cell-virus associations that may serve as systems for transduction. Tumor viruses would seem to be the best candidates for such a system, and for mammalian cells, viruses from mammalian tumors would be the first choice.

The polyoma virus, originally isolated from mice, produces a variety of tumors in different species (Stewart, Eddy, and Borgese, 1958, Eddy *et al.* 1959, Sachs *et al.* 1959, Fogel and Sachs, in press) and it can be grown and studied *in vitro*. In addition to its tumor inducing action, polyoma can act like a lytic virus and produce a cytopathic effect. This cytopathic effect on cells has been used to develop a plaque assay for this virus (Sachs, Fogel, and Winocour, 1959, Winocour and Sachs, 1959) in order to undertake quantitative studies on cell-virus relationships and to produce genetically pure lines of virus. Under optimum conditions the virus can be grown to a titer of 5×10^8 plaque forming units per ml. of culture fluid.

When polyoma virus is inoculated onto cultures of mouse embryo cells and produces its cytopathic effect, there are some survivors in the cell population. By culture of these survivors, a line of cells has been established that has been releasing virus for 9 months, and which has a stable cell-virus association (Medina and Sachs, in press). Mouse tumor cells, produced by inoculation of the virus into animals, also seem to have some form of stable cell-virus association. When such tumor cells are placed in culture, they can release virus (Sachs *et al.*, 1959, Sachs and Winocour, 1959), and when tested as single cells in microdrops, it was found that there was under these conditions at most only a low virus release per day (Krim and Sachs, in press). An example of the results with tumor cells is given in Table 5.

TABLE 5. CELL-VIRUS RELATIONSHIP IN MOUSE PAROTID TUMORS INDUCED BY POLYOMA VIRUS

Virus release per day from mass culture seeded with 5×10^6 tumor cells.

Days in culture	2	3	4
Total hemagglutination titer in tissue culture medium	8,192	16,384	8,192

Virus release from single tumor cells in microdrops during a 28 hour period.

No. of PFU per cell	0	1	2	3	4	5	6	7	8	9
No. of cells	10	0	1	2	2	1	1	1	0	1

This virus could thus be a good candidate for the establishment of a transducing system, and experiments on the *in vitro* induction of a stable cell-virus association are being continued in order to obtain an experimental system in mammals similar to that which has been developed for the Rous chicken tumor virus (Temin and Rubin, 1958). Polyoma may also be adaptable to human cells, and if successful, the use of a tumor virus in conjunction with antigenic or biochemical markers should prove to be a fruitful approach for genetic studies.

The development of new technics, as has been shown in this symposium, is making it possible to develop new approaches to the study of the genetics of man. We trust that the points that we have discussed may be of value in determining the choice of markers and experimental systems in order to extend our knowledge of the genetics of human cells.

REFERENCES

Atwood, K. C., and Scheinberg, S. L. 1958. Somatic variation in human erythrocyte antigens. *J. Cellul. Physiol.* 52: Suppl. 1, 97–115.

Coombs, R. R. A., Bedford, D., and Rouillard, L. M. 1956. A and B blood-group antigens on human epidermal cells demonstrated by mixed agglutination. *Lancet* i: 461–463.

Danon, M., and Sachs, L. 1957. Sex chromosomes and human sexual development. *Lancet* ii: 20–25.

Eddy, B. E., Stewart, S. E., Stanton, M. F., and Marcotte, J. M. 1959. Induction of tumors in rats by tissue culture preparations of SE polyoma virus. *J. Nat. Cancer Inst.* 22: 161–171.

Eichwald, E. J. and Silmser, C. R. 1955. Discussion of skin-graft data. *Transpl. Bull.* 2: 148–149.

Feldman, M., and Sachs, L. 1958. Immunogenetic properties of tumors that have acquired homotransplantability. *J. Nat. Cancer Inst.* 20: 513–539.

Fogel, M., and Sachs, L. The *in vitro* and *in vivo* analysis of mammalian tumor viruses IV. Antibody response and tumor-induction with polyoma virus in different species. *J. Nat. Cancer Inst.* (in press)

Gorer, P. A. 1956. Some recent work on tumor immunity. *Advance. Cancer Res.* 4: 149–186.

Gorer, P. A. and O'Gorman, P. 1956. The cytotoxic activity of isoantibodies in mice. *Transpl. Bull* 3: 142–143.

Klein, G., and Klein, E. 1958. Histocompatibility changes in tumors. *J. Cellul. Physiol.* 52: Suppl. 1, 125–168.

Koprowski, H., Theis, G., and Love, R. 1956. Immunological tolerance in tumour studies. Adaptation of ascites tumours to homologous or heterologous hosts. *Proc. R. Soc., Ser. B.* 146: 37–57.

Krim, M. and Sachs, L. Virus release from single cells of mouse parotid tumours. *Nature* (in press)

Lederberg, J. 1958. Genetic approaches to somatic cell variation: Summary comment. *J. Cellul. Physiol.* 52: Suppl 1, 383–401.

Medina, D., and Sachs, L. The *in vitro* formation of a stable cell-virus association with polyoma virus. *Virology* (in press).

Mitchinson, N. A. 1956. Antigens of heterozygous tumours as material for the study of cell heredity. *Proc. R. Phys. Soc. Edinb.* 25: 45–48.

Puck, T. T. 1958. Growth and genetics of somatic mammalian cells *in vitro*. *J. Cellul. Physiol.* 52: Suppl 1, 287–311.

Sachs, L. and Feldman, M. 1958. Cytotoxic antibodies in the homograft reaction. *J. Nat. Cancer Inst.* 21: 563–578.

Sachs, L., Feldman, M., and Danon, M. 1956. Prenatal identification of blood-group antigens. *Lancet* ii: 356.

Sachs, L., Fogel, M., and Winocour, E. 1959. *In vitro* analysis of a mammalian tumour virus. *Nature* 183: 663–664.

Sachs, L., Fogel, M., Winocour, E., Heller, E., Medina, D. and Krim, M. 1959. *In vitro* and *in vivo* analysis of mammalian tumor viruses. I. *Brit. J. Cancer* 13: 251–265.

Sachs, L., and Gallily, R. 1956. The chromosomes and transplantability of tumors III. The transplantability of mouse tumors with different degrees of strain specificity into previously immunized mice. *J. Nat. Cancer Inst.* 16: 1083–1097.

Sachs, L., and Heller, E. 1958. The sex-linked histocompatibility antigens. *J. Nat. Cancer Inst.* 20: 555–561.

Sachs, L., and Winocour, E. 1959. The formation of different cell-virus relationships in tumour cells induced by polyoma. *Nature* 184: 1702–1704.

Stern, C. 1957. The problem of complete Y-linkage in man. *Am. J. Human Genet.* 9: 147–166.

Stewart, S. E., Eddy, B. E., and Borgese, N. 1958. Neoplasms in mice inoculated with a tumor agent carried in tissue culture. *J. Nat. Cancer Inst.* 20: 1223–1243.

Temin, H. M., and Rubin, H. 1958. Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in the tissue culture. *Virology* 6: 669–688.

Welshons, W. J., and Russel, L. B. 1959. The Y chromosome as the bearer of male determining factors in the mouse. *Proc. Nat. Acad. Sc.* 45: 560–566.

Winocour, E. and Sachs, L. 1959. A plaque assay for the polyoma virus. *Virology* 8: 397–400.

Cell Cultures for Human Genetic Studies and Concluding Remarks[1]

M. FRACCARO

Institute of Medical Genetics, University of Uppsala, Sweden

As the last speaker of this symposium I will venture beyond my stipulated task of commenting on Dr. DeMars' excellent treatment of basic genetic studies with cell cultures and try to underline some general aspects of the human cell culture field.

From Dr. Chu's and Dr. Ford's papers we have learned how human or, gengenerally speaking, mammalian cytogenetics has overcome the technical difficulties which hampered progress for such a long time. Spectacular advances have been made possible by the manipulation of somatic cells in vitro. The potential simplicity of most of the methods used is, to my mind, the best antidote for that air of magic which has for a long time surrounded cell culture.

We have followed the logical and linear development from Dr. Barr's fundamental discovery of characteristic chromocenters in the resting nucleus to the direct observation of the human and mouse sex chromosomes. The first consequence of interest to all biologists will be the revision of the chapter on sex determination in the textbooks of genetics.

Dr. Sachs has shown us how wide is the field of application of the study of somatic cell variation.

From Dr. DeMars' paper we have learned of the rapid advances in the basic studies designed to answer fundamental questions on the nature of the genetic material, and of the promising future of these researches. For basic studies like those discussed by DeMars, any type of cell can be used (I personally would very much favor the mouse cell) provided it is stable enough in karyotype, metabolism, and nutritional requirements, conditions that are far from being fulfilled in most of the established strains now available. Incidentally, I feel that many of us are very demanding in this respect, probably more than microbiologists were at the time their important discoveries started the recent revolution in genetics. I quite agree with the idea, implicit in DeMars' remarks, that by being too demanding now we may hamper progress that can be sustained only with a reasonable degree of flexibility. Thus, we should not be more critical of the estimates of mutation rates recently obtained with somatic cells in culture (Lieberman & Ove, 1959) than we have been with the estimates of mutation rates

[1] Part of a symposium on Cytology in Human Genetics held on Sept. 2, 1959, under the joint sponsorship of the American Society of Human Genetics and the Genetics Society of America during the meetings of the American Institute of Biological Sciences at Pennsylvania State University, Aug. 30 to Sept. 3, 1959.

of "pathological" genes in human populations; no doubt the former are based on more direct evidence.

Human geneticists are also using cell culture, or plan to do so, with the hope of relating the findings in vitro to observations made on the living individual. If information is required on the action at the cellular level of specific genes selected for study in the living individual, the use of "primary" cultures is of paramount importance. This is linked with the problem of cell differentiation in vitro. We know that, with the rather uncontrolled conditions of primary cultures, cells carry out the functions of the tissue of origin for very short periods or not at all. Information on this point is still scanty and disorganized, though the literature on these aspects of tissue culture is enormous and spread over a period of more than 25 years. It is obvious on the other hand that we may look for and possibly find characteristics displayed in vitro that are peculiar to cells obtained from affected individuals even if quite different from those observed in vivo. Inborn errors of metabolism are obvious candidates for such studies and we hope that the ingenuity of clinicians and biochemists will help us to choose the right conditions for study. Personally, I consider the field of drug resistance and sensitivity a favorite candidate.

We should not forget that what we can observe both in vitro and in vivo is simply what the methods at our disposal allow us to see. Stern (1958) stressed this point at a recent symposium on somatic cell variation. He observed that when diploid cells carry a strictly recessive mutation in only one member of a pair of alleles, phenotypic expression must wait for some phenomenon like mutation of the other allele, somatic crossing over, etc. But the classification of a gene as "strictly recessive" depends upon the resolving power of our observational methods. When these are sensitive enough we may detect the effect of a gene in single dose in cultures of diploid cells as we detect it in the living individual in several traits in man. Here the so-called "biochemical" traits will probably attract the most attention, but the lesson of the sex chromatin should warn us not to forget any possible character, certainly not the morphological ones easily detected by simple microscopical observation.

To return to the problem of cell differentiation in vitro, the period of time in which the cells taken from the body still "remember" their original function seems to vary according to the wide range of experimental conditions in which they are kept. Recently Billen (1958) determined the persistence of bone marrow cells as such by their capacity to produce recovery in recipient lethally irradiated mice, and found that this was around ten days when the cells were cultured at 37° C., but around 20 days when cultured at 25° C. Reisner (1957) had previously obtained the record of 14 weeks by culturing the cells in suboptimal growth conditions. I think that a number of observations can be performed even in the short interval between the inoculation of the cultures (which in these cases can be massive) and the first transplant; the latter generally brings about a high degree of dedifferentiation, probably as a result of selective phenomena in the cell population.

We should also not ignore the old technique of "organ" culture by which

functional activities of explants may be preserved in vitro for quite long periods (Borghese, 1958).

In my experience, mainly with human fetal tissues, cells obtained from different organs have shown a high degree of morphological individuality in the first days of culture. This is often seen in the macroscopical appearance of the cell aggregates, even those formed by the re-aggregation of cells after dispersion with trypsin or similar dispersing agents. For example, cultures obtained by mechanical mincing of fetal brain display two types of cells, both probably of glial origin, clearly distinguished by their difference in type of outgrowth and size; these types persist even after several passages. Such differences are not of course a criterion of genetic properties and, as Stern (1958) has pointed out, a specific differentiation during development might be independent of, and superimposed upon, a constant genotype.

On the other hand, Norryd (1959) has recently described a human cell strain, derived from a parotid gland, which displayed two clearly distinct cell types of epithelial-like and fibroblast-like morphology in nearly equal proportions. The strain showed at the same time two stemline chromosome numbers of 79 and 73, also in nearly equal proportions. More observations of this type would certainly contribute a great deal to a better understanding of the relationship between genotype and phenotype of somatic cells.

Another instance of individuality is the observation made in our laboratory that cells derived from different fetal tissues (e.g. brain cortex and lung) have in vitro different rates of "spontaneous" chromosomal aberrations, in our case bridges and fragments scored at post-metaphase.

Morphological criteria are clearly not sufficient for our purposes and need to be integrated with other observations on the metabolic and nutritional characteristics of the different cell types.

This calls to mind the important observation recently reported by Hauschka (1958) that in Ehrlich ascites tumors the polyploid cells contained twice as much of cytochromes *b* and *c* as corresponding diploid cells, but content per unit volume was identical. Similar results were obtained by Patterson and Podber (1956) for aminopeptidase values. The ascites tumor is a special case not strictly comparable with our cultures, but correlated observations of these types should be performed whenever possible, even though it may seem difficult to have tht biochemist and the cytologist in the same place looking at the same cell population at the same time. In short, what we need is information on the natural history of our cultures taking into account as many factors as possible. Whenever a process puzzles us it should be investigated. As Eagle pointed out at a recent symposium in Napels, we are faced with differences in behavior which we can hardly explain: chromosome counts and enzyme spectra may change in unpredictable ways and morphological patterns have a similar behavior. For example, one Hela culture become fibroblastic in appearance and was then able to hydroxylate phenylalanine to tyrosine, but this ability was lost after some months. Other cultures may display a surprising constancy of behavior. Yet if we overlook details, mammalian cells are nearly uniform in their

inability to develop special functions, probably because, to use a rather meaningless sentence, they are too busy growing. These patterns are observed in established cell lines and I think they may be better understood if we follow our cultures carefully from the biopsy to this stage.

One may object that the study of primary cultures of cells obtained from individuals with known hereditary diseases is physiopathology of the diseases rather than genetics, and that it is futile to try to understand the action of specific selected genes before we have a better idea of the basic nature of the genic material. But whether we like it or not, human genetics is becoming more and more linked with the medical profession and I think that many of us, in my opinion rightly, will continue along these lines. But we must be prepared to go through the tedious job of performing and correlating an enormous mass of different technical procedures, many of which are costly in time and unrewarding in immediate results.

And now a word on techniques. We are still in the period in which techniques are varying and improving so rapidly as to make comparison among different experiments rather difficult. They are, as a matter of fact, as numerous as the laboratories working with cell cultures. No doubt there is in many quarters a curious tendency to overemphasize small technical details which are proved by subsequent experiment to be irrelevant. Tissue culturists are often superstitious about their cultures and what "makes them go". The very fact that the chromosome complements obtained in our laboratory (Böök *et al.*, 1959; Fraccaro *et al.*, 1959) are quite comparable with those, for example, of Dr. Chu and Dr. Ford, and that we are using techniques which differ in several details, shows that these details are not relevant at all. I stress this point because I want to make clear that cell culture is often all as easy as many other methods and with the enormous mass of work awaiting us we need to encourage people to use it and not hinder them with alleged technical difficulties.

The technical side is however important, as in any experimental science. Technical advances recently made by workers like Pomerat, Eagle, Dulbecco, and Puck (Paul, 1959) have been fundamental and have in a short period really revolutionized the field of tissue culture. But flexibility in application is what really brings out the best in any empirical technique. I may quote at this point a great British scientist who recently emigrated to India, where he hopes that "the lack of complicated apparatus may even stimulate us to look at what is before our eyes".

REFERENCES

Billen, D. 1958. The potentialities of tissue cultured cells of hemic origin in X-irradiated mice, in Anderson Hospital & Tumor Institute's *Radiation Biology and Cancer*, 223–237. Austin: University of Texas Press.

Böök, J. A., Fraccaro, M. & Lindsten, J. 1959. Cytogenetical observations in mongolism. *Acta paediat.*, Upps. 48: 453–468.

Borghese, E. 1958. Organ differentiation in culture, in McElroy, W. D. & Glass, B. (eds.). *A Symposium on the Chemical Basis of Development*, 704–773. Baltimore: Johns Hopkins Press.

FRACCARO, M., KAIJSER, K. & LINDSTEN. J. Somatic chromosome complement in continuously cultured cells of two individuals with gonadal dysgenesis. *Ann. Human Genet.* (in press)

HAUSCHKA, T. S. 1958. Correlation of chromosomal and physiologic changes in tumors. *J. Cellul. Physiol.* 52, Supp. 1, 197–233.

LIEBERMAN, I. & OVE, P. 1959. Isolation and study of mutants from mammalian cells in culture. *Proc. Nat. Acad. Sc.* 45: 867–872. Estimation of mutation rates with mammalian cells in culture. *Proc. Nat. Acad. Sc.* 45: 872–877.

NORRYD, C. 1959. The chromosomes of three human cell strains. *Hereditas* 45: 449–460.

PATTERSON, E. K. & PODBER, E. 1956. Aminopeptidase content as correlated with chromosome constitution in sublines of the Ehrlich ascites tumor. *Ann. N. York Acad. Sc.* 63: 988–997.

PAUL, J. 1959. *Cell and Tissue Culture.* Baltimore: Williams & Wilkins; London: E. & S. Livingstone.

REISNER, E. H. 1957. Tissue culture of bone marrow. *Clin. Res. Proc.* 5: 145 (abstr.).

STERN, C. 1958. The nucleus and somatic cell variation. *J. Cellul. Physiol.* 52, Supp. 1, 1–34.

BOOK REVIEWS

Maladies Héréditaries du Metabolisme chez l'Enfant

By Maurice Lamy, Pierre Royer and Jean Frézal, with a preface by Professor Robert Debré. Paris: Masson et Cie., 1959. 259 pp. 3,600 fr.

This timely book will be of particular interest to French-reading pediatricians and others interested in the genetics and clinico-pathological aspects of hereditary metabolic diseases of children. The introductory chapter gives a brief, clear discussion of the structure and function of the gene, since, in the words of the authors, "it is impossible to unravel the web of facts [about hereditary diseases] if one does not understand the concepts." Concepts of DNA structure, reproduction and mutation, gene recombination and pseudoallelism, genes and macromolecule synthesis, pleiotropy, dominance, penetrance and expressivity, are presented. Segregation ratios, Mendelian patterns in human pedigrees, the Hardy-Weinberg law, and methods of analysis in human genetics, are not dealt with, and it is clear from the following chapters that the authors assume that the reader is familiar with these subjects.

The authors make a number of intriguing suggestions, e.g. that the frequency of consanguinity in the parents of galactosemic children is low because some affected children represent double (trans) heterozygotes for pseudoalleles. On the other hand, this reviewer sees little point in suggesting that the genes for hemophilia A and B may be pseudoalleles, since they seem to be quite unrelated in their mode of action.

The rest of the book deals with specific hereditary metabolic diseases of children. Since it was impossible to include all such diseases, and still "keep the work within reasonable dimensions", the authors' choice of diseases was dictated by their personal experience and by their desire to present a wide panorama of the divers varieties of metabolic disease. The following categories are treated: galactosemia; the glycogenoses; anomalies of aromatic amino-acid metabolism (phenylpyruvic idiocy, alcaptonuria); adrenal hyperplasias; tubular transport anomalies (cystinuria, renal diabetes); vitamin D-resistant rickets (hypophosphatemia, hypophosphatasia); Toni-Debré-Fanconi syndrome and cystinosis; the diabetes insipidus's; mucoviscidosis; the sphingolipidoses (Gaucher's, Niemann-Pick's, amaurotic idiocy); gargoylism, idiopathic hypoglycemia; diabetes mellitus.

In general, each disease is dealt with according to its clinical picture, pathology and biochemistry, genetics and treatment. Unfortunately, there is no index, but each chapter has a good bibliography of selected references covering the period up until early 1958. The authors present an authentic and well-rounded picture of the state of knowledge of the diseases covered, and add many useful observations of their own.

F. C. Fraser
McGill University

Genetik des Menschen: Lehrbuch der Humangenetik

By Professor Otmar Freiherr von Verschuer. Munich: Urban and Schwarzenberg, 1959, pp. 427. d.m. 48.

This new textbook represents a considerable extension and revision of Professor von Verschuer's earlier textbook on "Erbpathologie" last published in 1945. In 356 pages

von Verschuer attempts the difficult task of giving a complete survey of the general principles of human genetics as well as of the specific genetics of normal traits and of all hereditary diseases. In contrast to the 1945 book, there is little space devoted to the applications of human genetics. The abolition of the Nazi laws on sterilization, whose implementation was fully discussed in the earlier book, does not fully explain this omission. In view of the author's earlier statements about the "superior value" of the "Nordic Race" and the danger of race mixture, one would like to find his present day views on practical eugenics and race more extensively discussed.

Knowledge of modern population genetics is becoming increasingly important for an understanding of human genetics. It is therefore surprising that the Hardy-Weinberg Law is not mentioned by name, although the author refers to its general principles in small print. Selective distribution of some genes is an important genetic phenomenon. When discussing pentosuria and familial dysautonomia, the author fails to point out that the genes responsible for these conditions practically only occur in populations of European-Jewish extraction.

Although modern concepts of biochemical genetics are mentioned, recent developments in heterozygote detection by biochemical means are only spottily covered. Diseases such as agammaglobulinemia are only listed and not discussed. The genetic heterogeneity of methemoglobinemia and thalassemia are not pointed out. The statement that occurrence of a given trait in both father and son excludes sex linked inheritance (p. 43) needs qualification as only applying to rare traits.

In contrast to many other books on human genetics, there is full and adequate discussion of normal human traits. The author rightly indicates that normal traits rarely are monogenic, and tabulates the minimum number of loci for a variety of characteristics such as hair form (2), skin color (3), and ear shape (3). Some geneticists might object that without knowledge of the detailed developmental genetics of such traits, such tabulations are premature and possibly misleading.

The author's tabulation of dominant, recessive, and sex linked genes for each organ system totalling 412 gene loci represents the first attempt at a quantitative estimate of known mutant genes in man. In view of the variable source of the data, much caution is necessary in the use of this estimate.

The author's treatment of the specific disease groups is necessarily uneven since full, authoritative, and accurate coverage of all of genetic medicine is impossible by any one individual at this time. The chapter on structural abnormalities of bone, teeth, and connective tissue is especially complete. The physician needing specific information on a given disease will find the book a useful introduction with extensive literary citations for further work. In spite of its limitations, this textbook will be of value to medical students and physicians with genetic interest. It will be helpful as a reference work for genetic clinics. Professor von Verschuer deserves the gratitude of all those interested in human and medical genetics for having summarized and brought up to date a great deal of scattered information.

The book has many illustrations and pedigrees and is well printed.

Arno G. Motulsky
Department of Medicine
University of Washington

LETTERS TO THE EDITOR

O. Vogt and the Terms "Penetrance" and "Expressivity"

September 28, 1959

To the Editor.
Dear Sir:

On July 31st, 1959, Oscar Vogt died, at the age of 89. The main field of Vogt's research was neuroanatomy but he also contributed to facts and interpretations in what has become known as "The New Systematics." When he founded the Kaiser Wilhelm Institut für Hirnforschung in Berlin-Buch, he included in it a section of Genetics to which he called Timoféeff-Ressovsky, who had been trained in the U.S.S.R. and had just begun his scientific work there. In a paper on "psychiatrically important facts of zoologic-botanic taxonomy" published in the Zeitschrift für die gesamte Neurologie und Psychologie in 1926 (101: 805–832) Vogt coined two words which have become part of the terminology of genetics, and particularly of human genetics: penetrance and expressivity. In discussing experiments of Romaschoff's and Timoféeff-Ressovsky's on *Drosophila funebris* Vogt wrote:

Es gibt Genenvariationen, welche sich unter den verschiedensten Bedingungen durchsetzen. Man muss diese Tendenz zum Sichdurchsetzen scharf von der Dominanz trennen. Ich schlage vor, diese Tendenz zum Sichdurchsetzen als "Penetranz" zu bezeichnen....

...Von Penetranz und Spezifizität ist dann noch eine dritte, von Timoféeff unterschiedene Eigenschaft der Genenvariation zu trennen, für welche man die Bezeichnung "Expressivität einführen kann. Bei starker Penetranz, d.h. bei prozentual häufiger Manifestierung eines Merkmals kann dieses in schwacher Form auftreten (schwache Expressivität). Umgekehrt kann bei schwacher Penetranz, d.h. bei prozentual seltener Manifestierung eines Merkmales dieses bei seinem Auftreten in sehr ausgesprochener Form in Erscheinung treten (starke Expressivität). Auch diese Expressivität ist durch den Rest des Keimplasmas beeinflussbar....

The fact that it was Vogt who invented these terms is confirmed by H. A. and N. W. Timoféeff-Ressovsky in a paper published in 1926 in Roux' Arch. f. Entwicklungsmechanik (*108*, p. 150, footnote 1) and again by N. W. Timoféeff-Ressovsky in 1929 in the Journal für Psychologie und Neurologie (*38*, p. 137). The related term "specificity" seems first to have been used by the Timoféeffs in the above named footnote of their joint publication of 1926.

It is of historical interest that the terms penetrance and expressivity were introduced by an investigator whose main work was related to medicine.

CURT STERN
Department of Zoology
University of California
Berkeley 4, California

Ectopia Lentis et Pupillae

September 15, 1959

To the Editor.
Dear Sir:

In the June number of Vol. 11 of this periodical WALLS and HEATH claim to have found "a 'new' dominant gene for ectopia lentis et pupillae of variable expressivity, isolated from Marfan's syndrome and unaccompanied by 'predisposition to rheumatic heart defects.'"

In my opinion the variable expressivity makes it difficult to decide whether the authors are right. In their pedigree I meet with two difficulties:

1°. the first affected generation counts 3 affected females, among whom are dizygotic twins and one unaffected brother. This high number of affected siblings in one family, issue of non-

affected parents, who have no diseased relatives, does not necessarily compel us to accept dominance of the trait, because this can also happen in recessive inheritance. If it were dominance, then it should be irregular dominance by lack of penetrance in one of the parents. It might also be an instance of germinal mosaicism. The lack of information on the direct ascendents during a number of generations makes it impossible to solve this problem.

2°. dominance has been assumed because one of the affected twin sisters, who showed the complete syndrome of ectopy of the lenses and pupils, has a son with a unilateral incomplete syndrome: a slightly ectopic lens. It is true, that there was a bilateral tremulous iris, but both pupils were normal. Is this a sufficient base for the assumption of dominance? I hesitate, because the twin sister of the mother of the boy only showed bilateral ectopic, spontaneously-luxated lenses and the other maternal aunt a unilateral ectopic lens like the boy. This variable expressivity or, better formulated, variable penetration of one of the symptoms, is well-known in this syndrome, but we do not know whether the individuals with insufficient expressivity and lack of penetrance are heterozygotes or homozygotes, in other words: whether occasionally the mode of inheritance may be intermediate. It is therefore that I must recommend the authors to investigate families of this kind in all directions, especially the following generations. If later on the incompletely affected boy should marry a non-related girl and should get a child with the complete syndrome, then the dominance would be proved.

This question made me read the publications of STREBEL (1914) and STREBEL and STEIGER (1914) again. The titles suggest a dominant ectopia lentis et pupillae associated with heart defects. This would be unique indeed. However I am not sure that congenital ectopic pupils regularly belonged to the clinical picture. Their presence in some of the patients is not striking. There is only one drawing of the iris of one patient. The pupil was triangular, very little dislocated and the picture was made after luxation of the lens to the anterior chamber. The description in 3 unilaterally affected members is insufficient and the story of 2 others is from hearsay. At any rate it is quite uncertain whether there were dominant ectopic pupils. Repeatedly the authors mention the ectopic lenses and only incidentally an ectopic pupil. One photograph of a male patient makes it probable (deep lying eyes, long hands) that we deal with a pedigree of Marfan's syndrome. It is uncertain whether the incidental ectopic pupil(s) were primary or secondary.

My purpose is not to invalidate the conclusion of WALLS and HEATH, that they have found a new dominant gene for ectopic pupillae et lentis, but to warn for too rapid statements on a too small material. We must be well aware of the relative value of mendelian terms in modern genetics (see Gordon ALLAN, p. 365 of his periodical, September 1958).

P. J. Waardenburg,
Hazegrietje 29, Arnhem

ot
re
s.
ct

ne
n-
s,
I
c,
e
e
h
er
re
e-
a
e

d
d
al
-
s
e
e
-
y
g
-

e
-
n